Final Report

For the Project

DEVELOPMENT OF BIOLOGICAL INDICATORS OF NUTRIENT ENRICHMENT FOR APPLICATION IN TEXAS STREAMS

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Prepared by:

Ryan. S. King, Ph.D.
Principal Investigator and Project Contact
Associate Professor, Department of Biology, Baylor University
One Bear Place #97388, Waco, TX 76798
Tel: 254.710.2150; E-mail: Ryan_S_King@baylor.edu
Lab webpage: www.baylor.edu/aquaticlab

and

Kirk O. Winemiller, Ph.D.
Co-Principal Investigator; Texas AgriLife Research, Texas A&M University

Co-investigators:
Jason M. Taylor, Ph. D. candidate (King), Dept. of Biology, Baylor
Jeffrey A. Back, Ph.D. candidate (King), Dept of Biology, Baylor
Allison Pease, Ph. D. candidate (Winemiller), Texas A&M University

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The TCEQ has committed to the development of nutrient criteria for waters in Texas as presented in the November 3, 2006 draft of the Nutrient Criteria Development Workplan. Under that plan the TCEQ is exploring several complementary strategies to develop nutrient criteria. Strategies now being investigated include the following: 1) basing criteria on concentrations of nutrients; 2) basing criteria on direct indicators of eutrophication, such as chlorophyll a; 3) developing "translator" procedures that relate concentrations of nitrogen and phosphorus to direct indicators of eutrophication; 4) basing criteria on historical "ambient" averages with a statistical allowance for variability; and 5) developing criteria based on the effect of nutrients or indicators of eutrophication on uses.

This study is only one component of the larger water quality standard criteria development process that will involve a diverse stakeholder workgroup and formal public participation process to establish regulatory criteria. For this study the choice of data analysis, presentation and interpretation of results, and the report conclusions and recommendations are those of the Principal Investigators and not those of the Texas Commission on Environmental Quality (TCEQ). No official endorsement of the TCEQ should be inferred.

GENERAL DESCRIPTION OF THE SPECIAL STUDY

Water quality criteria and standards are developed by States to improve water quality and to ensure that a water body is supportive of its designated aquatic life uses (ALU). Nutrient pollution caused by excessive loading of nitrogen (N) and/or phosphorus (P) can significantly limit the ability of streams to support their designated ALUs. In response to this problem, the U.S. EPA published its National Strategy for the Development of Regional Nutrient Criteria (1998), which detailed a comprehensive plan to be used by States for the development of scientifically defensible, numerical criteria for nutrients. The plan emphasized the need for the inclusion of endpoints that reflect the biological integrity of aquatic ecosystems and are supportive of ALUs. However, relatively few States have been sufficiently equipped with the necessary nutrient and biological data that could be used to develop defensible regional criteria, particularly for wadeable streams. Consequently, many States are either struggling to develop criteria or have had to implement numerical nutrient criteria based on regional EPA guidance without a clear understanding of the implications of these criteria to supporting biological integrity and ALUs in their respective States.

Texas has made significant progress in the development of nutrient criteria for reservoirs, but limited research has been done in wadeable streams. Because streams function differently than reservoirs, indicators of nutrient-related degradation that are used in reservoirs may have limited applicability in streams (e.g., surface-water chlorophyll a). In streams, attached vegetation (algae, bacteria, fungi, and macrophytes) and associated animals (macroinvertebrates, fish) are the biological indicators most likely to be affected by nutrient enrichment. Identifying linkages between surface-water nutrients (e.g., total phosphorus, total nitrogen) and biological indicators of aquatic life uses in streams is therefore imperative for development of defensible, numerical nutrient criteria.

We evaluated new indicators of nutrient-related alteration to wadeable stream ecosystems by bridging two complementary ongoing projects in the Subhumid Agricultural Plains (SAP) ecoregion of Texas. (1) a TCEQ-funded study on refinement and validation of habitat quality indices (HQI) and fish Index of Biotic Integrity in 64 streams in the SAP ecoregion, directed by

K. O. Winemiller of Texas A&M University (TAMU) with R. S. King of Baylor University (BU) (Winemiller et al. 2009), and (2) a US EPA Region 6 funded study on nutrient criteria development in the Cross Timbers subregion of the SAP of Texas, directed by R.S King at BU (Appendix B). At the initiation of the EPA study, BU intentionally selected 26 of their TCEQ HQI sites for their EPA study with the goal of maximizing physical, chemical and biological information for criteria development. However, sites sampled by TAMU located in the Blackland Prairies and East Central Texas Plains ecoregions of the SAP have not been sampled for nutrients or other nutrient indicators, as well as some additional HQI study sites sampled by BU in the Cross Timbers. Thus, we expanded sampling of surface-water nutrients and a few key biological indicators (periphyton metrics) to the 12 additional HQI sites not sampled for nutrients by BU and all 26 sites studied by TAMU as part of the TCEQ HQI study, thus adding nutrients and periphyton to the ongoing habitat and fish assessments at 64 stream sites in summer 2008. The linkage between the EPA and TCEQ studies effectively added a considerable amount of information for relatively little cost because the additional sample collection took place during site visits already planned for fish and habitat assessment. This overarching goal of the study was to help Texas in its ongoing effort to develop defensible, effects-based nutrient criteria in wadeable streams.

The specific objectives of this study were:

1) Compare surface-water total phosphorus (TP) and total nitrogen (TN) values measured by Baylor University with values measured by TCEQ. BU and TCEQ labs differ in minimum detectable limits (MDL) between methods for TP, and use different methods altogether for TN;
2) Evaluate the utility of soft-substrate periphyton (episammon/epipelon) as an indicator of nutrient enrichment in soft-bottomed streams of the Texas Blackland Prairies and E. Central Texas Plains, and contrast responses of perihyton to nutrients in these ecoregions with those of the Cross Timbers (epilithon, or rock-substrate periphyton).
3) Estimate thresholds, if present, in periphyton (nutrient content, biomass, and species composition) and other biological variables measured in the habitat assessment (e.g., microalgae/biofilm cover, macrophyte cover) in response to surface-water TP, TN, and other indicators of stream condition such as sedimentation (e.g., mud-silt cover, substrate

embeddedness), as well as the potential drivers of these stressors (pasture, rowcrop, impervious cover, WWTP outfalls);

4) Evaluate responses of fish communities to nutrient enrichment, sedimentation, and drivers of those stressors;
5) Recommend responsive ecological indicators and identify nutrient concentrations (thresholds) that correspond to changes in ecological indicators for potential use in nutrient criteria development and stream assessments.

STUDY AREA AND STREAM SAMPLING

Study area

Data were collected from 64 wadeable streams in the Brazos and Trinity River basins within the Cross Timbers, Blackland Prairies, and East Central Texas Plains ecoregions (Figures 1-3). The Cross Timbers ecoregion (ECO 29) is a mosaic of forest, woodland, savanna, and prairie and is currently used mostly for rangeland and pastureland. The Texas Blackland Prairies ecoregion (ECO 32) is a disjunct ecological region distinguished from neighboring regions by fine-textured, clayey soils. This region was historically tallgrass prairie and now contains a higher percentage of cropland than adjacent ecoregions. In addition, large areas of the ecoregion are being converted to urban and industrial uses. The East Central Texas Plains ecoregion (ECO 33) was historically covered by post oak savanna and now is used primarily for pasture and rangeland (Griffin et al. 2004).

Watershed variables describing physical characteristics and topography, land use, and distribution of hydrologic disturbance points (outfalls and dams) were calculated for each site. Watershed boundaries for each sample site were automatically digitized in ArcGIS 9.2 with the ArcHYDRO 9 extension using a 1:24,000 scale digital elevation model (DEM) expressed as a 30 m raster, available from the U. S. Geological Survey. Mean slope and elevation were calculated for each watershed using the digital elevation model. Mean annual precipitation was calculated for each watershed from a polygon coverage of average monthly and annual precipitation. This dataset was obtained from USDA-NRCS. Number of wastewater outfalls and cumulative permitted outfall discharge (MGD) were calculated for each watershed based on the TCEQ municipal and industrial wastewater outfall shapefile available from http://www.tceq.state.tx.us/gis/sites.html. Landcover class percentages were calculated for each watershed using National Land Cover Database (NLCD 2001) available from http://www.mrlc.gov/nlcd_multizone_map.php. All watershed analyses were performed with ArcGIS 9.2 (ESRI, Redlands, CA.).

We sampled the 64 streams in the summer (June, July, and August) of 2008. At each stream site, a 160-500 m study reach was designated for periphyton and water chemistry sampling, fish collection and local habitat measurements. Reach length was determined based upon the wetted width of the stream (approximately 40 times the average width). Study reach selection, fish collection, and habitat measurements were performed following the protocols of TCEQ Surface Water Quality Monitoring Procedures (TCEQ 2003, 2004).

Local-scale environmental variables

At each study site, we measured 57 habitat variables (Appendix A1) including substrate composition, instream cover, wetted width, depth, canopy cover, bank slope, riparian buffer width, dissolved oxygen (instantaneous), conductivity, and pH on the same dates as fish sampling. We made these measurements at 5 to 6 evenly spaced transects (depending on reach length). Some measurements, such as number of riffles, maximum pool depth, stream sinuosity, and composition of riparian vegetation, were summarized for the entire study reach. Discharge (in ft^3/sec) was also measured along a representative transect within each reach using a portable electromagnetic flow meter (Marsh-McBirney Flo-Mate Model 2000). These variables and their relation to the HQI and fish communities were analyzed in Winemiller et al. (2009). A few of these variables were examined carefully in this study because they were shown to be related to fish communities in Winemiller et al. (2009), and of their potential as indicators of nutrient or nutrient-related stressors (e.g., microalgae cover, macrophyte cover, substrate embeddedness, mud-silt cover).

Water chemistry and periphyton sampling

Water chemistry sampling consisted of two sets of surface-water instantaneous grab samples and one reach-scale composite of epilithic (removal and compositing of periphyton from surface of at least 25 rocks, if present) or episammic (composite of several fixed-area samples of sand or finer sediments) periphyton.

The first set of surface-water grab samples for total phosphorus (TP) and total nitrogen (TN) analysis at BU were collected in triplicate in accordance with BU's EPA-approved project QAPP. The second set of surface-water grab samples for TCEQ Houston Laboratory analysis of total kjeldahl nitrogen (TKN), nitrate-nitrite-N (NO3-NO2-N), ammonia-nitrogen (NH3-N), total phosphorus, orthophosphate-P (PO4-P), seston chlorophyll-*a* (CHLA), total alkalinity, chloride, total suspended solids, volatile suspended solids, sulfate, total dissolved solids, fluoride, and total phosphorus were sampled in accordance with TCEQ Surface Water Quality Monitoring Procedures Volume 1. All field sampling was accomplished by BU and TAMU investigators. In accordance with the applicable procedures and QAPPs, all samples were preserved where necessary, stored at 4°C upon collection, and shipped to BU and TCEQ, respectively, within 24 hours in coolers. The periphyton samples were collected in accordance with the BU nutrient study QAPP.

Periphyton composite samples were handled and analyzed in accordance with the project QAPP. All periphyton physical and chemical analysis was conducted by BU. Periphyton was shipped to BU on ice (4°C) within 24 hours of collection. Periphyton was homogenized and aliquots of known volume were analyzed for the following: total carbon (C), N, and P in the organic (OM) fraction of the periphyton (%); C, N, P per unit dry mass of bulk periphyton (no separation into OM or sediment fractions); ash-free dry mass (AFDM) (g/m2); chlorophyll a (mg/m2); and cell densities of the algae species in the periphyton (no/cm2). Periphyton OM fractions were separated from the bulk (unfractionated) periphyton by suspending aliquots in colloidal silica and centrifuging the mixture to separate sediment or other heavy particles from the lighter algae, bacteria, and other organic matter. Following centrifugation, the OM fraction was rinsed to remove colloidal silica, dried at 60ºC for 24 h, pulverized to a fine powder, and analyzed for C, N, and P following Back et al (2008) and Scott et al (2008).

Algae species samples were homogenized, preserved, and identified in accordance with taxonomic methods for soft and diatom algae described in TCEQ (2005). One soft and one diatom taxonomic sample was identified per stream per year. At least 500 diatom and 300 soft algae cells per respective sample were identified (TCEQ 2005). Dr. Barbara Winsborough, an expert periphyton taxonomist from central Texas, performed all of the species identifications in accordance with the approved project plan.

Fish sampling

Within each study reach, all available habitats were sampled using a backpack electrofisher (Smith-Root Model LR-24) and seine net (15' x 6' or 6' x 6'). Crews of 3-4 people electrofished each study reach in a single upstream pass with a minimum effort of 900 seconds. The reach was then sampled with a seine net with a minimum of six 10-m hauls. Sampling continued beyond the minimum effort until all habitats were sampled and no new species were captured within the study reach. Collected fishes were identified, separated into juvenile and adult age classes, counted, and either released into the habitat or preserved in 10% buffered formalin for later identification. A detailed description of fish community composition and important environmental correlates among the 64 sites is included in Winemiller et al. (2009).

Figure 1. Map showing the study region in the Brazos and Trinity watersheds. Colored lines delineate ecoregion boundaries (green = Cross Timbers, brown = Texas Blackland Prairies, yellow = East Central Texas Plains).

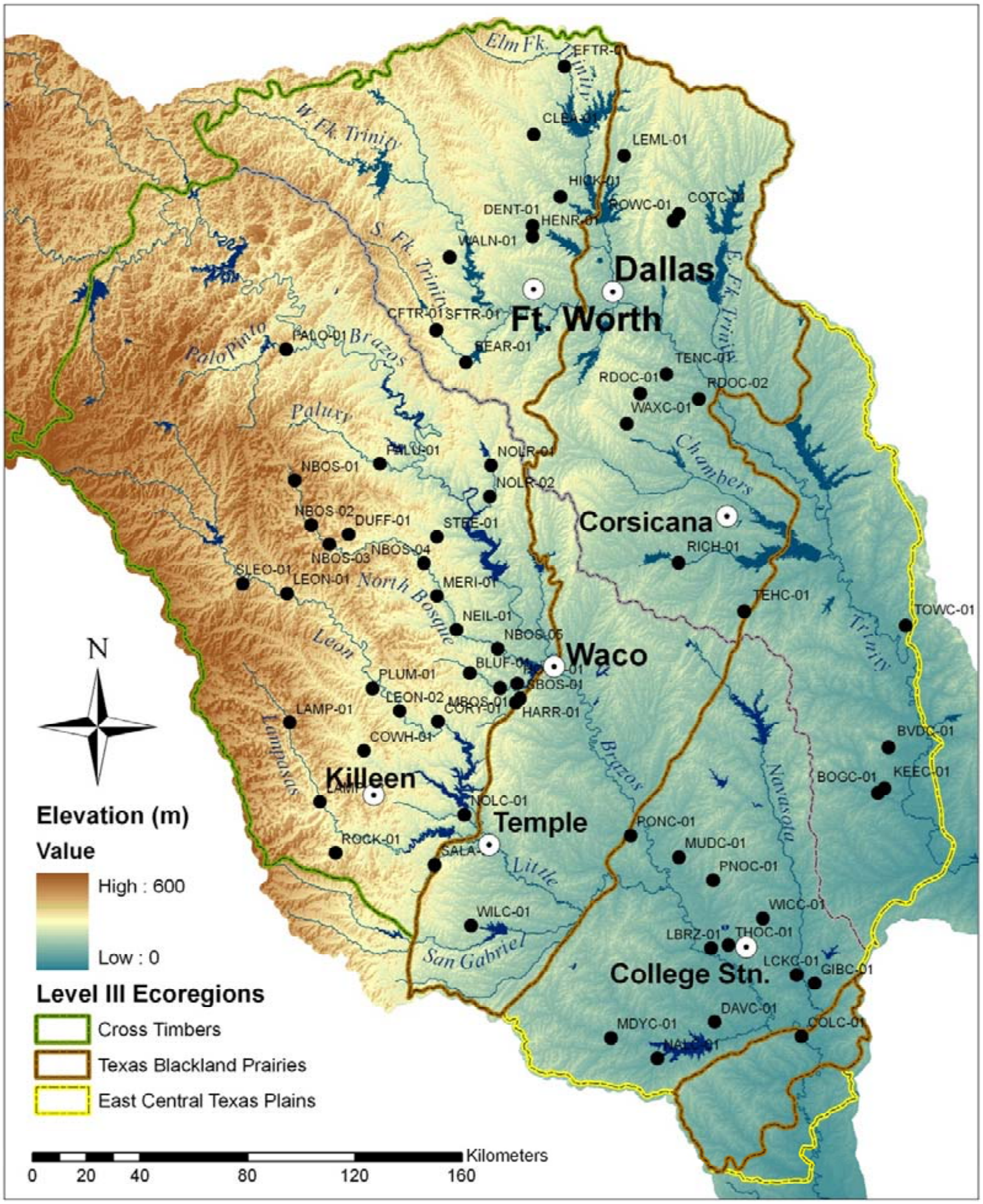

Figure 2. Map showing the 64 study sites and elevation gradients across the Trinity and Brazos basins and the three ecoregions within the SAP.

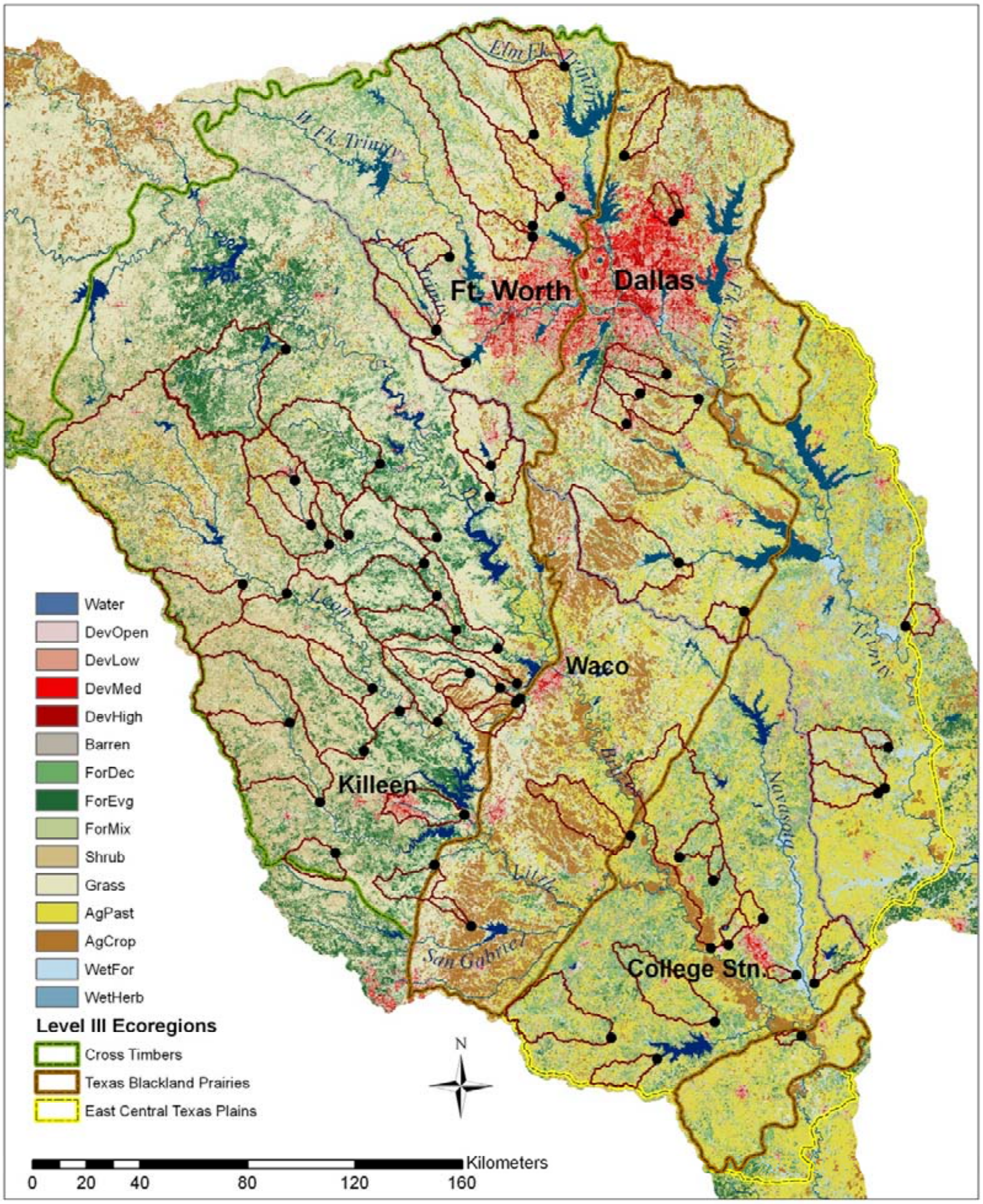

Figure 3. Spatial distribution of dominant land-cover classes among the 26 study watersheds (NLCD 2001).

DATA ANALYSES

We followed the analysis framework outlined by King and Richardson (2003) and the aforementioned EPA Region 6 study (King et al. 2009; Appendix B) to identify variables that were candidate indicators of nutrient-related reductions in biological integrity. Nutrient and response variable data were graphically evaluated to initially screen variables and data sets for relationships that could be reasonably analyzed using threshold statistical techniques, as biological responses to nutrients were likely to be nonlinear and heteroscedastic. Conditional dot plots were used to examine distributions of variables among ecoregions, whereas lattice scatterplots were used to visualize and contrast stressor-response relationships.

We estimated potential threshold responses of univariate biological variables (e.g., periphyton nutrient ratios, chlorophyll a, macrophyte cover, etc) to numerical levels of nutrients or nutrient-related stressors using nonparametric changepoint analysis (nCPA), a technique explicitly designed for detecting threshold responses using ecological data (King and Richardson 2003, Qian et al. 2003). This analysis is based on the fact that structural change in an ecosystem may result in a change in both the mean and the variance of an ecological response variable used to indicate a threshold. **When observations are ordered along an environmental variable (gradient), a changepoint is simply the value that separates the data into the two groups that have the greatest difference in means and variances.** This can also be thought of as the degree of within-group variance relative to the between group variance, or *deviance* (*D*). Analytically, the nCPA examines every point along the stressor gradient and seeks the point that maximizes the reduction in deviance.

There is one particular value of the predictor *y* (e.g, TP) that maximizes the reduction in deviance in the response data (in this case, the selected biological responses); however, there is uncertainty associated with that value. It is unlikely that any one value of the predictor (e.g., TP) is the only value that could represent a changepoint. In reality, depending on the acuteness of the biological change in response to TP, several observations of TP could represent the changepoint, each with varying probabilities. Thus, to assess the risk associated with particular levels of TP, nCPA incorporates estimates of uncertainty in the changepoint (King and Richardson 2003). These estimates are calculated using a bootstrap simulation. This simulation resamples (with replacement) the original dataset and recalculates the changepoint with each simulation. Bootstrap simulations are repeated 1,000 times. The result is a distribution of changepoints that summarizes the uncertainty among multiple possible changepoints. This uncertainty is expressed as a cumulative threshold frequency based on the relative frequency of each changepoint value in the distribution.

Multivariate algal and fish species abundance data were handled differently than the univariate biological data. First, important differences (gradients) in species composition and environmental correlates of those gradients were identified using non-metric multidimensional scaling (nMDS). NMDS is a distance based procedure that ordinates study units based on rank dissimilarities (Minchin 1987, Clarke 1993, Legendre and Legendre 1998). We used Bray-Curtis dissimilarity (BCD) as the distance measure, a coefficient that has been repeatedly demonstrated to be robust for ecological community data (Faith and Norris 1989). A two-dimensional solution was used for all analyses as stress values (a measure of agreement between BCDs and the configuration of the ordination) were relatively low and did not substantially decrease when additional axes were included in ordinations. Before running ordinations on the data sets, algae or fish species occurring at only two sites (algae) and one site (fish) within a data set were excluded, and abundances were log transformed. Algae and fish data matrices were analyzed separately. Variables from the watersheds and environmental measurements with high skewness (> 1) were also log transformed to improve linear relationships with the ordinations. Ordinations were performed in PC-Ord version 5.20 (MjM Software, Gleneden Beach, OR, U.S.A.).

We used rotational vector fitting to relate environmental and watershed variables to gradients in algal and fish community composition quantified by the NMS ordinations (Faith and Norris 1989). Vector fitting was used to find the direction of the maximum correlation for each environmental variable. Significance ($P \leq 0.05$) of each environmental vector was estimated using 1,000 random permutations of the data. Vector fitting was performed using the ECODIST package in R version 2.5.1 (© 2007, The R Foundation for Statistical Computing).

To estimate species thresholds to nutrients or other stressors identified in the ordinations, we employed a new analytical approach, Threshold Indicator Taxa ANalysis (TITAN; King and

Baker), with the goals of (1) exploring and identifying abrupt changes in both the occurence frequency and relative abundance of individual taxa along nutrient gradients, (2) quantifying uncertainty associated with both observed distributions of each taxon and the broader sample, and (3) estimating the relative synchrony of those changes as a non-parametric assessment of a community threshold. Current statistical methods used for grouping samples and detecting community ecological thresholds are not developed for distinguishing responses of individual taxa with low occurrence frequencies or highly variable abundances (Dufrêne and Legendre 1997, Brenden et al. 2008, Andersen et al. 2008). Some methods assume a linear, univariate response along all or part of an environmental gradient (e.g., Toms and Lesperance 2003), whereas others focus solely on aggregate, community-level dissimilarity (e.g., De'Ath 2002, King et al. 2005) or species turnover between samples (i.e., beta-diversity). Noisy, non-linear, and poorly distributed occurrences are typical properties of the vast majority of taxa in multivariate community data matrices (McCune and Grace 2002). Multivariate or multi-metric analysis can obscure distinct responses of taxa subsets in a community data set, especially if both predominant and rare species do not respond in a similar fashion or focal species do not respond as expected. TITAN circumvents these problems.

TITAN represents a combination and extension of change-point and indicator species analysis. In TITAN, we use normalized indicator species taxa scores (z) to identify the value of a continuous variable, x, resulting in the optimal partitioning of sample units, such that the indicator score is maximized either for individual taxa or the additive response of all normalized indicator z -scores at the community level. Negatively responding taxa (z–) are distinguished from those responding positively (z+) to yield taxa-specific change-point distributions as well as cumulative responses of declining [sum(z–)] and increasing [sum(z+)] subsets of the community. Resampling procedures are used to measure both indicator reliability and purity, and to assess estimate uncertainty surrounding the existence of community change-points.

TITAN analysis was performed on the same species data sets as in the ordinations using log-transformed abundance data. Predictors included important nutrient variables identified from the environmental vector fitting analysis. TITAN was conducted in R version 2.5.1 (© 2007, The R

Foundation for Statistical Computing) using the custom package TITAN written by M. E. Baker and R. S. King (Baker and King, in revision; King and Baker, in revision).

RESULTS AND INTERPRETATION

Comparison of BU and TCEQ TP and TN laboratory methods

Baylor and TCEQ total phosphorus (TP, ug/L) data corresponded closely above the TCEQ lab method detection limit (LOD) of 50 ug/L (Figure 4). Importantly, 34 of the 64 sites had TP concentrations below the TCEQ LOD, whereas all sites fell above the BU lab MDL of 3.6 ug/L (Appendix C, BU TP method). This result is particularly significant given the results of King et al. (2009; Appendix B) and new results reported in this document that provide compelling evidence of numerous biological changes in response to TP concentrations above 20 ug/L, a level well below the TCEQ LOD.

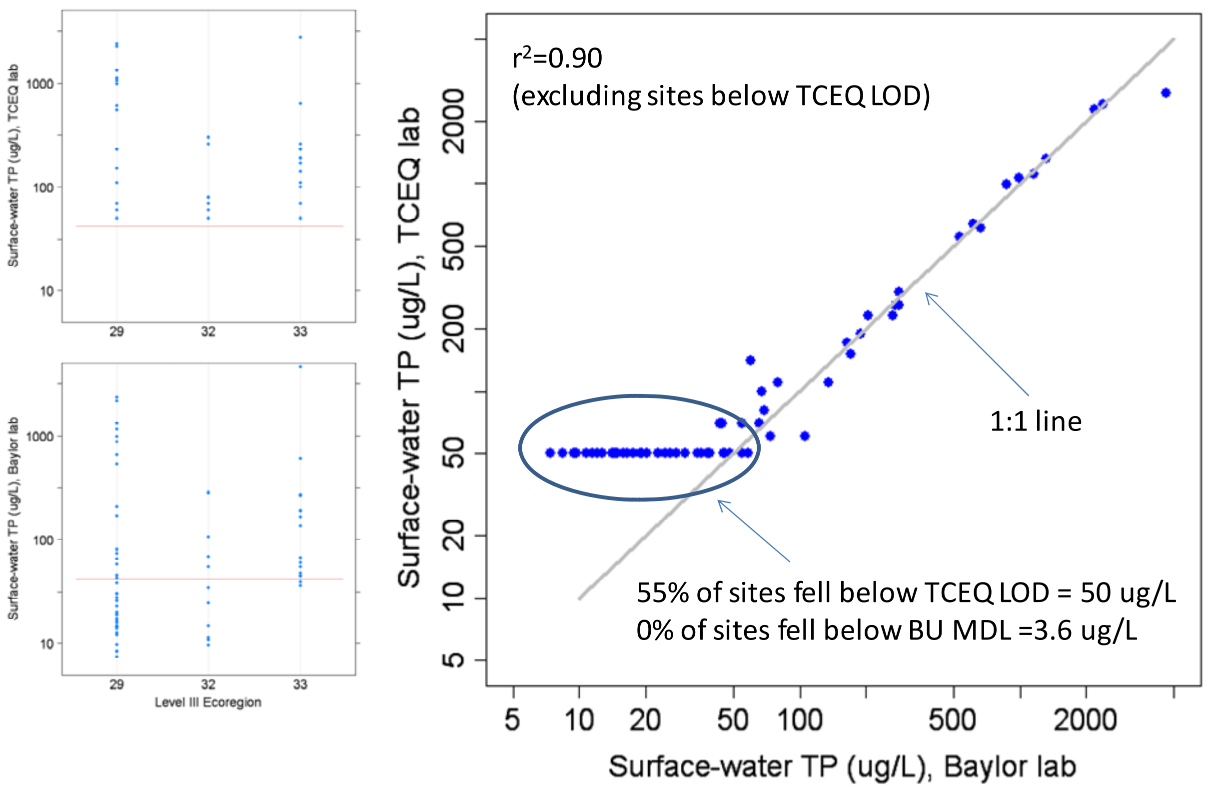

Figure 4. Distribution of surface water TP (ug/L) values among ecoregions (29=Cross Timbers, 32=TX Blackland Prairies, 33=E.Central.TX Plains) and analytical methods (BU=Baylor, TCEQ). The main panel (right) shows that above the TCEQ LOD of 50 ug/L, the two methods match quite well (r2=0.90, close to 1:1 correspondence), but over half of the streams in the study area fell below the TCEQ LOD. The two small panels (left) show that most of samples that fell below the TCEQ LOD (red line) were in Ecoregions 29 and 32.

The BU and TCEQ results for total nitrogen (TN) corresponded quite well for most of the distribution of values (Figure 5; r^2=0.91). Two discrepancies between methods were evident: 1) variance in the TCEQ TN data began to increase at the low end of the distribution, and 2) TCEQ values were consistently above the 1:1 line between methods. Both of these were likely due to the way the TCEQ TN value was computed in this report. TCEQ measures TN as total Kjeldahl nitrogen + nitrite-nitrate-N + ammonia-N, each with its own method and LOD. Baylor (BU) converts all forms of nitrogen by digestion (Appendix D) to nitrate-N and measures it with one method. Because some of the nitrogen components in the TCEQ methods fell below the method LOD, we assumed that the LOD was the measured value (we could not assume that it was zero), thus the sum of the nitrogen parameters typically included a LOD value that artificially elevated the TN estimate. It appears that, except for low levels of TN, the TCEQ and BU TN methods yield similar results and the TCEQ LODs may not be an important source of error in TN estimation.

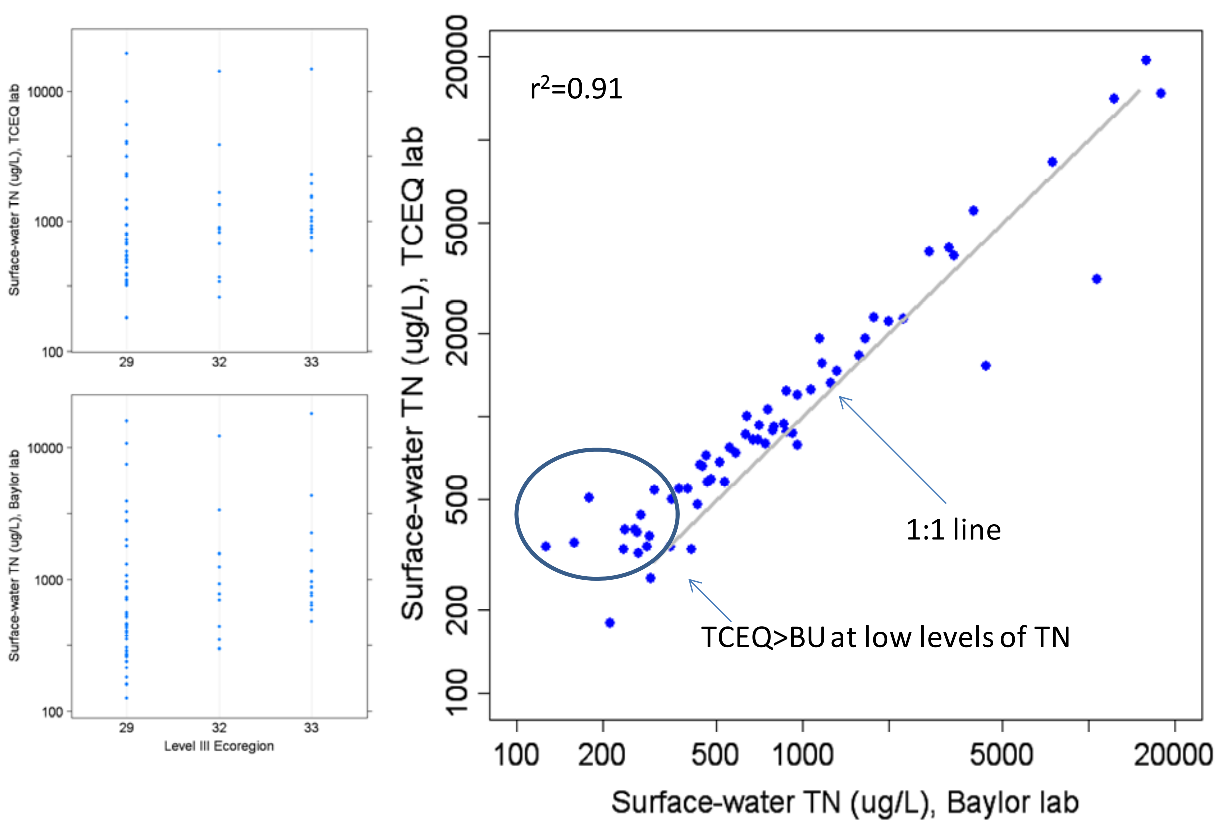

Figure 5. Distribution of surface water TN (ug/L) values among ecoregions (29=Cross Timbers, 32=TX Blackland Prairies, 33=E.Central.TX Plains) and analytical methods (BU=Baylor, TCEQ).

Surface-water TN and TP (hereafter, BU lab data for these two analytes) were positively correlated among the 38 sites in the Cross Timbers (Ecoregion 29) and the 15 sites in the East Central Texas Plains (Ecoregion 33), but no relationship was evident among the 11 sites from the Blackland Prairies (Ecoregion 32; Figure 6).

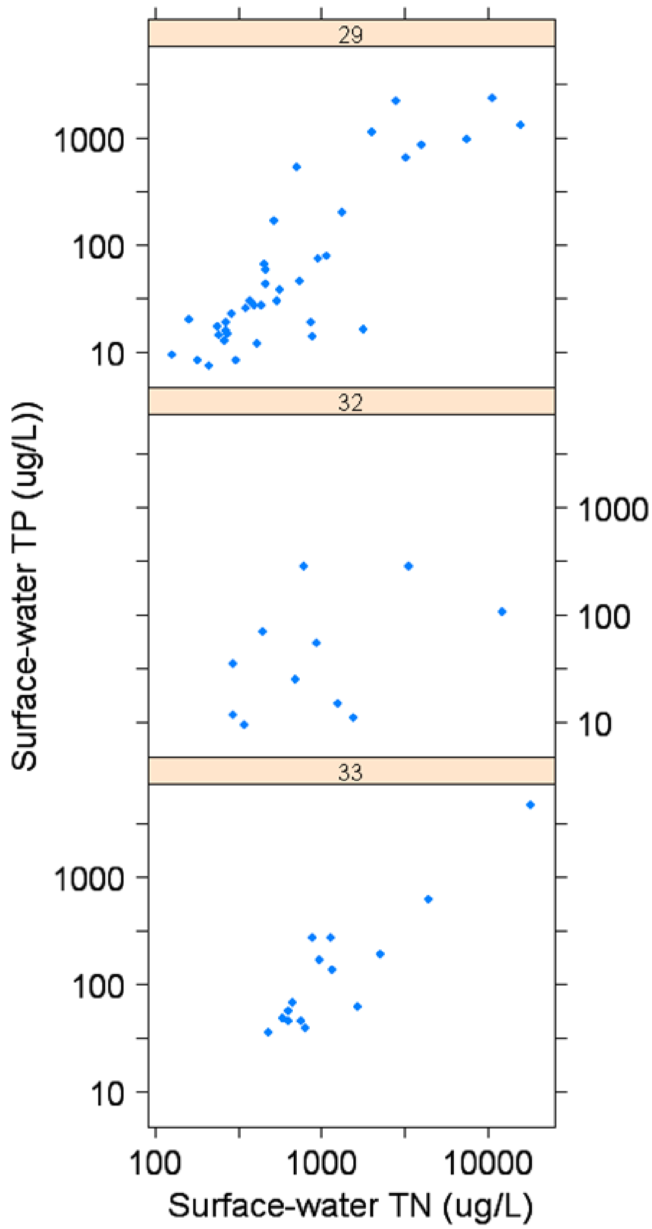

Figure 6. Scatterplots of TN vs. TP among the 3 ecoregions.

Ecoregion 29 had a wide range of TN and TP values, spanning a gradient from <10 and <100 ug/L to >2,000 and >15,000 ug/L TP and TN, respectively (Figure 6, top panel). There also were sites, particularly at low levels of TP, that tended to have relatively high concentrations of TN, which would potentially be important for evaluating whether P or N were more responsible for biological changes, if any were evident.

Ecoregion 32 had a much narrower range of TP than Ecoregion 29, with a few values near 20 ug/L and none above 500 ug/L TP (Figure 6, middle panel). With only 11 sites, and only 2 of those in the Brazos basin, coupled with the narrow range of TP values, statistical analysis of biological responses to nutrient gradients would yield results that would be uncertain and more likely to be confounded by other variables or outliers.

Ecoregion 33 had an even narrower of TN and TP values than Ecoregion 32, which is undesirable for characterization of biological responses to nutrients (Figure 6, bottom panel). All of the TP values in Ecoregion 33 were > 40 ug/L, much higher than the biological thresholds observed by King et al. (2009; Appendix B). Low sample size (n=15) likely resulted in an insufficient characterization of the distribution of nutrient levels in Ecoregion 33.

Periphyton nutrient content across ecoregions

Periphyton from rocks (Ecoregion 29) and sand/mud (Ecoregions 32, 33) had considerably different distributions of nutrient ratios (C:P, C:N, N:P, Figure 8). Not surprisingly, there was a much larger difference in ratios between the bulk and OM fractions in the sand/mud samples than rock samples, likely because of the much higher proportion of sediment to OM in these samples.

Figure 7. Photograph of a subsample of homogenized periphyton suspended in water (middle tube) following laboratory processing (bulk, or unfractionated periphyton), and four tubes containing aliquots of periphyton that were suspended in colloidal silica and centrifuged to separate the organic matter (algae, bacteria, detritus, fungi) from heavier, mostly inorganic particles (silt, clay, sand). The lighter organic material is pulled to the top of the suspension, whereas the sediment is pulled to the bottom during the centrifugation process. Following centrifugation, the organic fraction is removed using a pipettor, dried, pulverized, and analyzed for total carbon, nitrogen and phosphorus. The unfractionated bulk periphyton sample is dried, pulverized, and analyzed in the same manner but without separation from inorganic particles. See Appendix B for details.

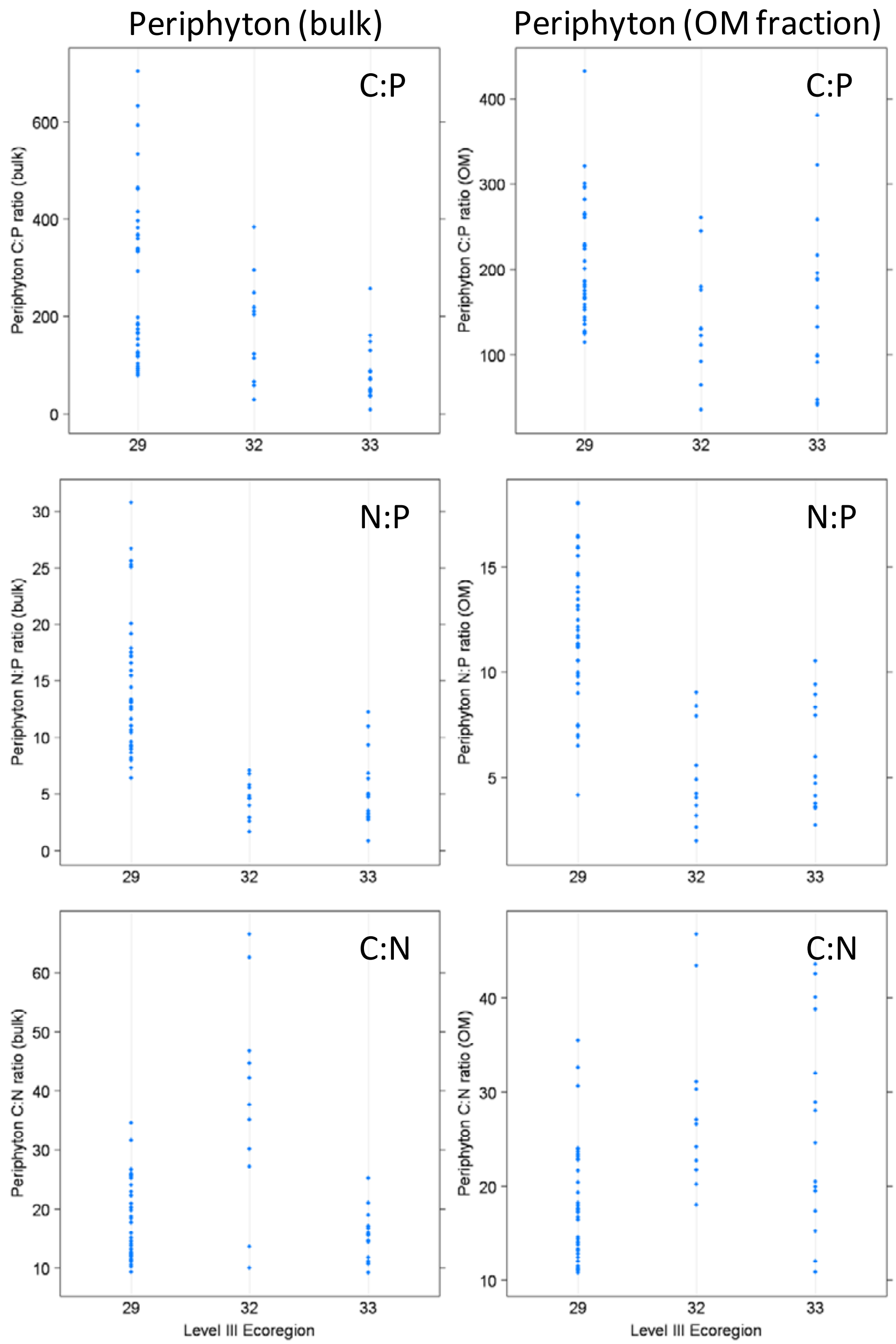

Figure 8. Distribution of C:P, N:P, and C:N ratios among streams in Ecoregions 29, 32, and 33 and between bulk and OM fractions of periphyton.

Rock periphyton (Ecoregion 29) nutrient ratios were strongly, and nonlinearly, related to surface-water nutrient concentrations (Figure 9, 10). Periphyton C:P and N:P ratios declined sharply with small increases in TP. The difference between OM C:P ratios and bulk C:P ratios was very high for periphyton in streams with low levels of TP, but rapidly diminished with TP enrichment. This implied that the bulk periphyton, which contained both sediment and the exopolysaccharide bacterial matrix, was storing as much phosphorus as the cellular organic matter (algae, fungi, bacteria). This was consistent with results of King et al (2009; Appendix B), reinforcing the strong connection between surface-water enrichment and rapid uptake, storage, and recycling of nutrients, particularly phosphorus, in the periphyton.

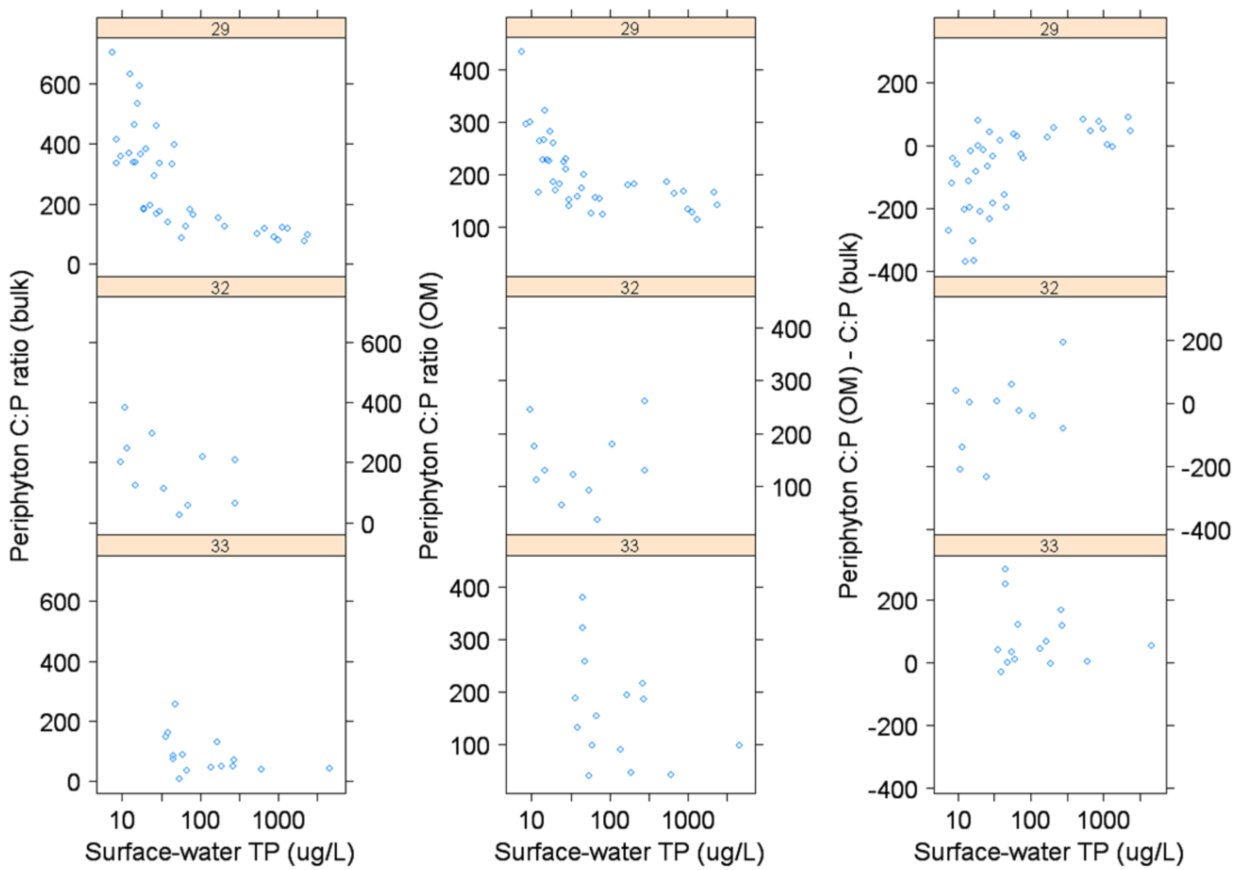

Figure 9. Scatterplots of periphyton C:P ratios (bulk, OM, and OM minus bulk) in response to surface-water TP across the three ecoregions.

Unfortunately, little of the variance in sand/mud periphyton ratios corresponded to surface-water nutrient concentrations (Figure 9, 10). Sand/mud C:P, C:N, and N:P ratios were unrelated to surface-water TP or TN in Ecoregion 32. There was a subtle relationship between C:P ratios and TP in Ecoregion 32, but the pattern was noisy and interpretation was difficult with so few samples.

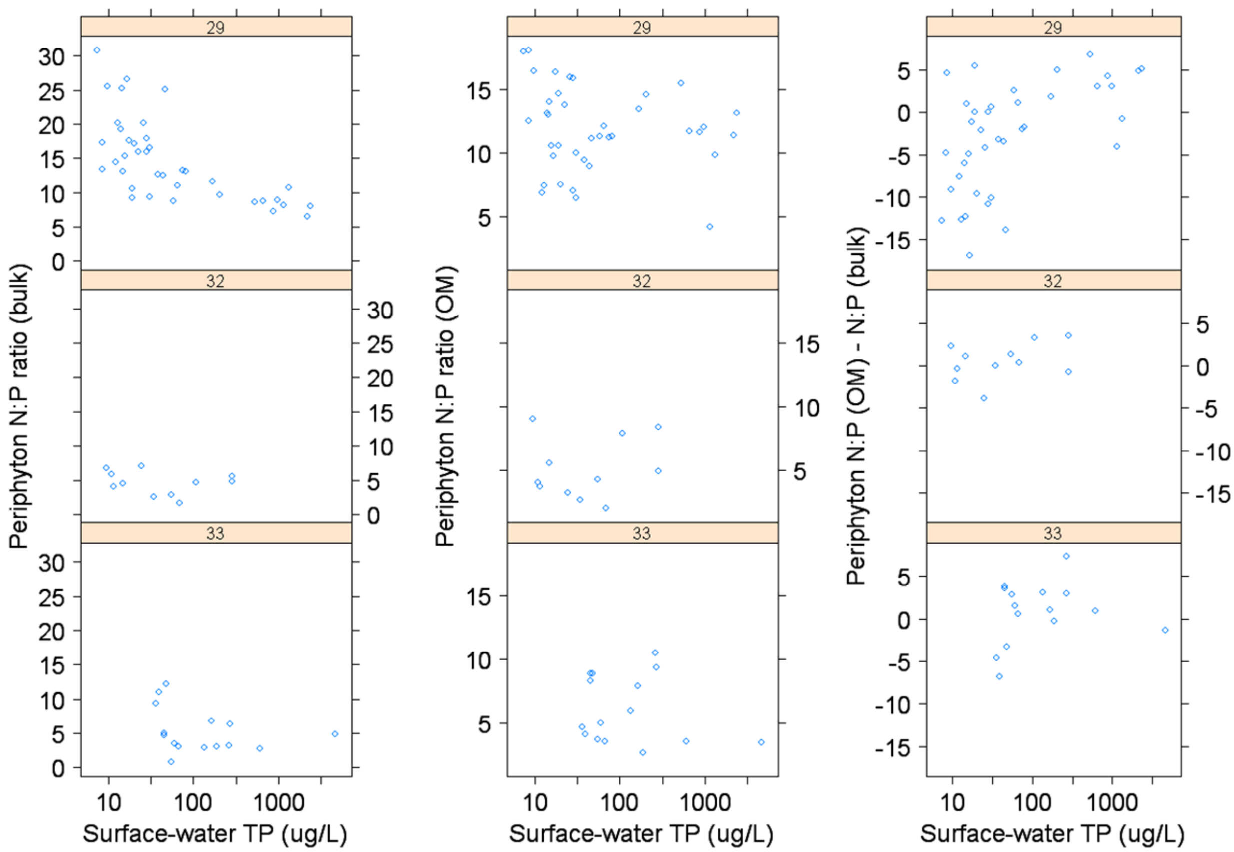

Figure 10. Scatterplots of periphyton N:P ratios (bulk, OM, and OM minus bulk) in response to surface-water TP across the three ecoregions.

RESULTS AND INTERPRETATION, CONTINUED

Surface-water and periphyton chlorophyll across ecoregions

Surface-water chlorophyll-a increased sharply in response to TP and TN in Ecoregion 29 (Figure 11, TP results only). Most of the sites with values below detection limits for chlorophyll were at low levels of TP. This pattern was not clear in Ecoregion 32 and 33, but was confounded by sample size and gradient length.

Periphyton chlorophyll-a per unit area trended toward a slight increase in Ecoregion 29, but was very noisy. However, the ratio of chlorophyll a to AFDM (ash-free dry mass) increased with TP, reflecting a shift from more calcareous periphyton to a community comprised of more filamentous and colonial green algae (King et al. 2009; Appendix B). Periphyton chlorophyll appeared to decline in response to TP in Ecoregion 32, whereas no relationship was evident in Ecoregion 33.

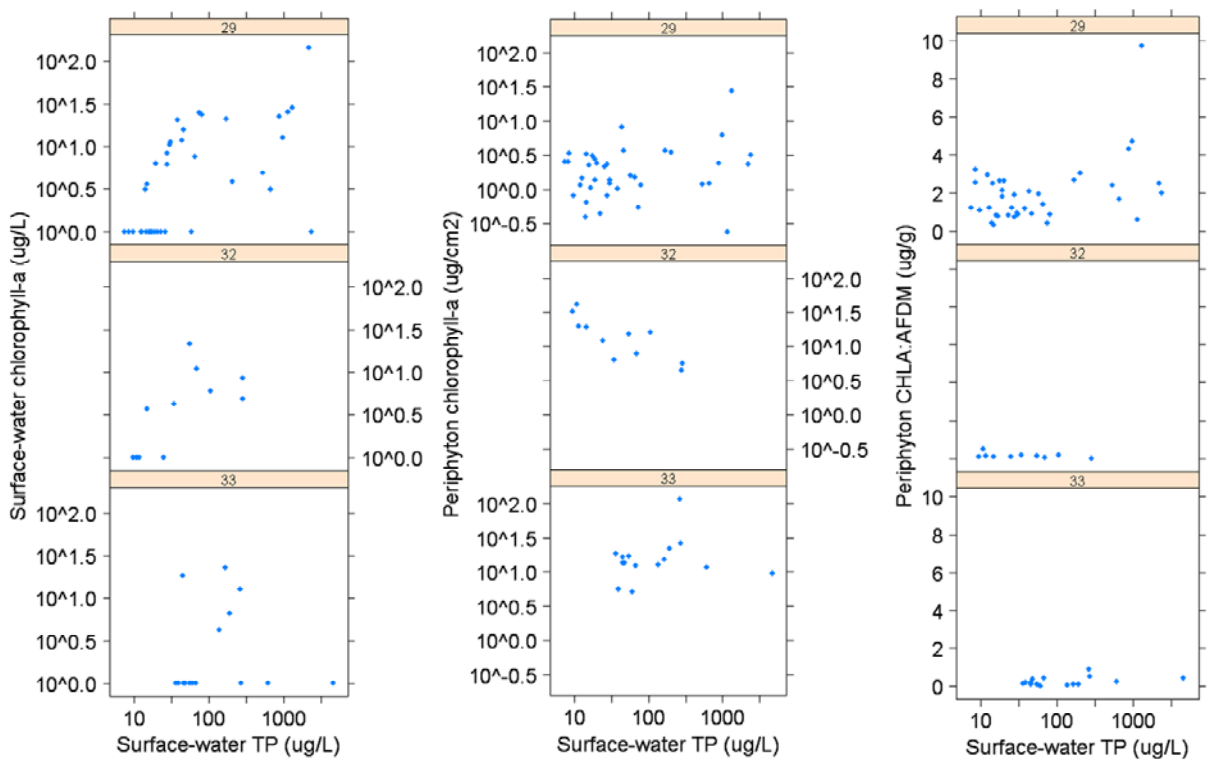

Figure 11. Scatterplots of chlorophyll-a (ug/L, water), chlorophyll-a (mg/m2, periphyton), and the ratio of periphyton chlorophyll-a to AFDM (mg/g) across the three ecoregions.

RESULTS AND INTERPRETATION, CONTINUED

Estimation of thresholds for univariate biological indicators, Ecoregion 29

Qualitative results revealed that insufficient sample sizes and noisy data rendered threshold analysis in Ecoregions 32 and 33 to be impractical. However, the large number of sites, graphically obvious nonlinear changes in several variables, and wide range of nutrient concentrations in Ecoregion 29 was suitable for statistical analysis of thresholds.

Surface-water chlorophyll-a and nonfiltrable residue showed very similar responses to TP in Ecoregion 29 (Figure 12; note that outlier for both variables did not influence the changepoint estimate). Both were near or below detection limits at TP<25 ug/L, showed a sharp, significant increase above 25 ug/L TP (Table 1). Both variables also increased significantly above a TN threshold of ~350 ug/L (Table 1).

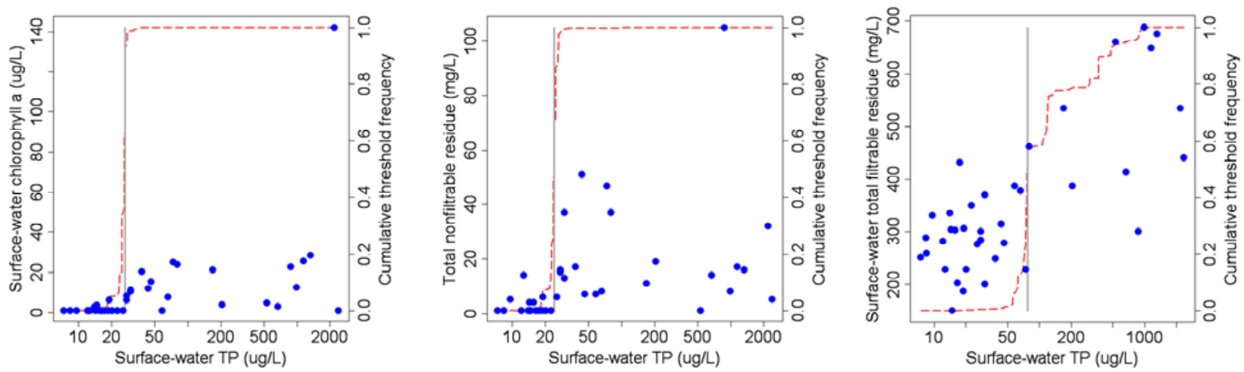

Figure 12. Results from nonparametric changepoint analysis using **surface-water TP** as a predictor of threshold changes in **surface-water chlorophyll-a, nonfiltrable residue, and filterable residue** in **Ecoregion 29**. Each blue dot represents one of the 38 sites sampled in summer 2008. The gray vertical line is the observed TP threshold (the level of TP resulting in the greatest difference in the response variable to the left and right of that value). The dotted red line is the cumulative threshold frequency, an estimate of uncertainty based on 1,000 bootstrap samples of the data (see King and Richardson 2003). The cumulative threshold frequency illustrates the range of possible threshold values; different quantiles of this distribution can be interpreted as confidence intervals around the observed threshold. See Table 1 for summary of the corresponding statistical results.

Total filtrable residue also increased significantly with increasing TP and TN (Figure 12; TP results only). However, its threshold level of TP and TN were less certain (Table 1).

Perphyton C:P, N:P, and C:N ratios sharply declined in response to TP (Table 1, Figure 13). Periphyton C:P (bulk) and C:P (OM) both declined significantly at <20 ug/L (Table 1, Figure 13), reinforcing periphyton C:P ratios as a very sensitive, robust indicator of nutrient enrichment in Cross Timber streams (King et al. 2009; Appendix B). The bulk samples also appeared to be nearly as sensitive to TP as the OM samples.

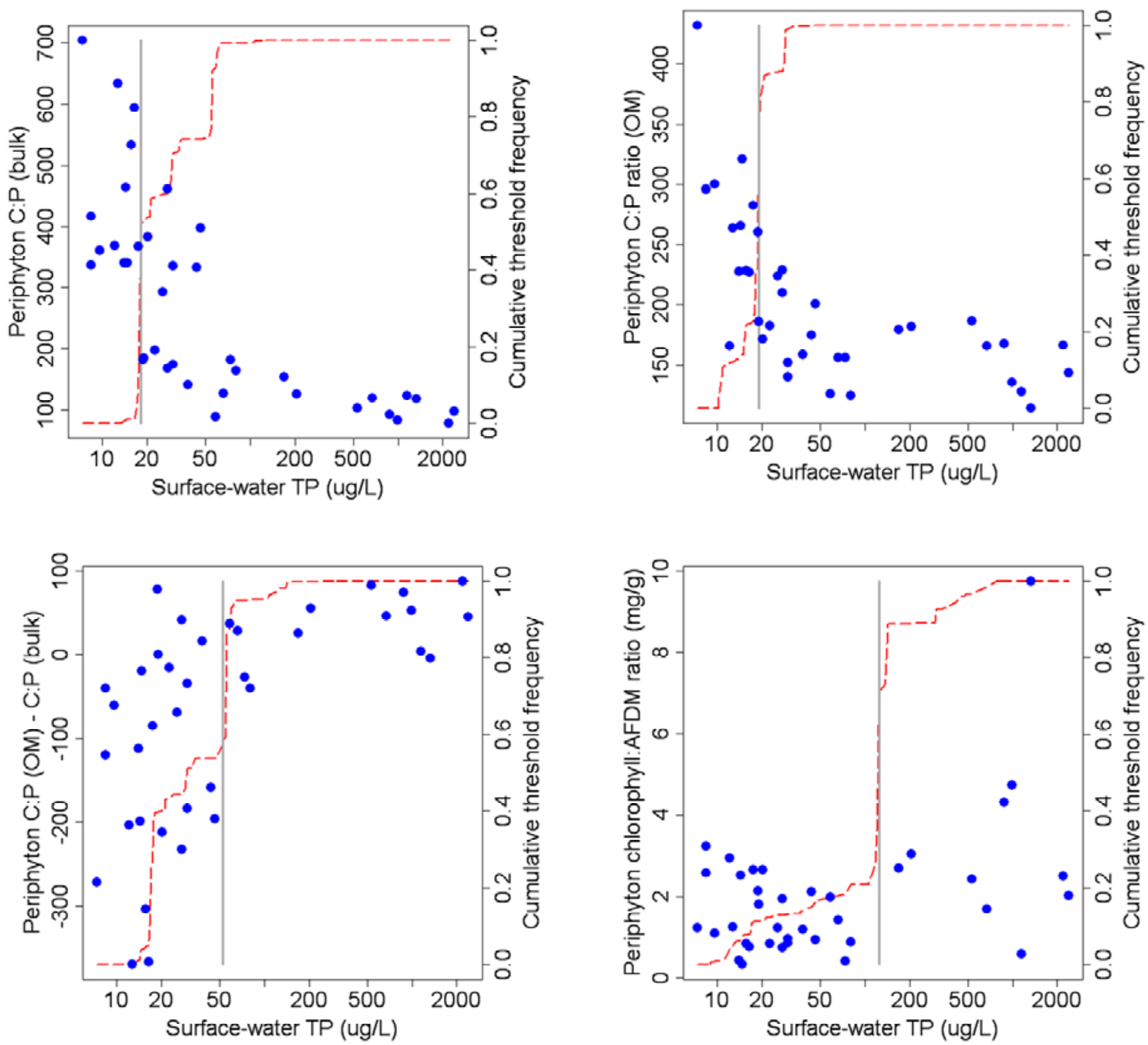

Figure 13. Results from nonparametric changepoint analysis using **surface-water TP** as a predictor of threshold changes in **periphyton variables** in **Ecoregion 29**. Each blue dot represents one of the 38 sites sampled in summer 2008. The gray vertical line is the observed TP threshold (the level of TP resulting in the greatest difference in the response variable to the left and right of that value). The dotted red line is the cumulative threshold frequency, an estimate of uncertainty based on 1,000 bootstrap samples of the data (see King and Richardson 2003). The cumulative threshold frequency illustrates the range of possible threshold values; different quantiles of this distribution can be interpreted as confidence intervals around the observed threshold. See Table 1 for summary of the corresponding statistical results.

Table 1.. Results of nonparametric changepoint analysis using nutrients and nutrient-related predictors of threshold responses in fish community indicators of biological integrity in Ecoregion 29. See figures 12 through 13 for graphical display of some of these results.

					Bootstrap threshold quantiles				
Predictor	Response	Response > threshold	Threshold (obs)	P value	10%	50%	90%	Mean<obs	Mean>obs
Total N (ug/L)	AFDM_M2	ns	280.17	0.5335	277.17	462.50	1661.17	1.36	1.08
Total N (ug/L)	**CHLA_UGL**	**Increase**	**362.00**	**0.0010**	**338.83**	**374.00**	**487.83**	**1.10**	**8.21**
Total N (ug/L)	**CHLA:AFDM**	**Increase**	**3246.67**	**0.0043**	**685.95**	**1660.00**	**2786.67**	**1.61**	**5.21**
Total N (ug/L)	CHLA_M2	ns	3246.67	0.0665	271.00	1140.50	2786.67	1.53	5.98
Total N (ug/L)	**C:N (OM)**	**Decline**	**440.83**	**0.0206**	**280.17**	**440.83**	**1143.17**	**21.23**	**15.24**
Total N (ug/L)	**C:N (BULK)**	**Decline**	**440.83**	**0.0061**	**277.17**	**440.83**	**511.00**	**21.71**	**14.41**
Total N (ug/L)	C:N OM-BULK	ns	1891.67	0.2213	261.83	468.25	1891.67	-0.38	3.01
Total N (ug/L)	**C:P ALG**	**Decline**	**362.00**	**0.0004**	**277.17**	**328.17**	**377.58**	**270.93**	**167.78**
Total N (ug/L)	**C:P BULK**	**Decline**	**266.00**	**0.0029**	**263.83**	**384.67**	**918.17**	**472.70**	**219.39**
Total N (ug/L)	**C:P OM-BULK**	**Increase**	**266.00**	**0.0186**	**263.83**	**284.50**	**1891.67**	**-192.74**	**-36.83**
Total N (ug/L)	EMBEDDED	ns	420.17	0.0506	280.17	420.17	918.17	25.78	45.41
Total N (ug/L)	**TFILRESI**	**Increase**	**1891.67**	**0.0039**	**440.83**	**1016.00**	**1891.67**	**314.00**	**528.57**
Total N (ug/L)	MACRPHYT	ns	362.00	0.1196	280.17	362.00	800.17	4.24	0.68
Total N (ug/L)	MCRPH_AB	ns	362.00	0.0607	295.67	374.00	918.17	0.54	0.15
Total N (ug/L)	MICRALG	ns	800.17	0.0630	295.67	792.67	807.67	9.86	0.41
Total N (ug/L)	**MUDSILT**	**Increase**	**328.17**	**0.0318**	**318.83**	**337.08**	**918.17**	**0.27**	**12.19**
Total N (ug/L)	**NFILRESI**	**Increase**	**328.17**	**0.0051**	**318.83**	**338.83**	**918.17**	**0.25**	**1.02**
Total N (ug/L)	N:P (OM)	ns	362.00	0.0615	238.83	362.00	414.33	13.72	10.91
Total N (ug/L)	**N:P (BULK)**	**Decline**	**261.83**	**0.0104**	**251.83**	**423.17**	**1891.67**	**21.39**	**13.08**
Total N (ug/L)	N:P OM-BULK	ns	2393.33	0.0560	261.83	445.83	2285.00	-3.87	3.31
Total P (ug/L)	AFDM_M2	ns	368.33	0.3764	12.44	69.78	368.33	1.23	0.90
Total P (ug/L)	**CHLA_UGL**	**Increase**	**26.78**	**0.0003**	**24.00**	**26.78**	**26.78**	**1.28**	**10.65**
Total P (ug/L)	**CHLA:AFDM**	**Increase**	**125.08**	**0.0291**	**17.03**	**125.08**	**303.10**	**0.10**	**0.43**
Total P (ug/L)	CHLA_M2	ns	770.33	0.3042	14.27	40.73	665.00	1.60	2.99
Total P (ug/L)	**C:N (OM)**	**Decline**	**30.18**	**0.0124**	**16.60**	**29.00**	**55.85**	**20.94**	**14.56**

Total P (ug/L)	**C:N (BULK)**	**Decline**	**21.43**	**0.0014**	**19.05**	**24.00**	**54.50**	**22.88**	**14.29**
Total P (ug/L)	C:N OM-BULK	ns	932.17	0.1869	13.42	26.78	703.67	-0.30	3.81
Total P (ug/L)	**C:P ALG**	**Decline**	**19.05**	**0.0002**	**10.89**	**19.05**	**28.95**	**274.22**	**166.07**
Total P (ug/L)	**C:P BULK**	**Decline**	**18.23**	**0.0004**	**17.77**	**18.32**	**55.85**	**454.67**	**188.74**
Total P (ug/L)	**C:P OM-BULK**	**Increase**	**52.08**	**0.0030**	**17.03**	**30.18**	**59.97**	**-129.82**	**33.50**
Total P (ug/L)	**EMBEDDED**	**Increase**	**21.43**	**0.0106**	**16.65**	**21.43**	**30.18**	**22.00**	**47.03**
Total P (ug/L)	**TFILRESI**	**Increase**	**77.03**	**0.0002**	**63.10**	**77.03**	**471.39**	**284.89**	**522.00**
Total P (ug/L)	MACRPHYT	ns	26.78	0.2288	16.18	26.78	69.78	3.45	0.64
Total P (ug/L)	MCRPH_AB	ns	26.78	0.0909	16.18	26.78	69.78	0.47	0.13
Total P (ug/L)	MICRALG	ns	15.30	0.1400	15.12	25.20	598.33	13.33	4.54
Total P (ug/L)	**MUDSILT**	**Increase**	**24.22**	**0.0088**	**23.02**	**24.22**	**25.35**	**0.67**	**14.07**
Total P (ug/L)	**NFILRESI**	**Increase**	**24.22**	**0.0003**	**22.38**	**24.22**	**26.78**	**0.24**	**1.17**
Total P (ug/L)	**N:P (OM)**	**Decline**	**10.89**	**0.0309**	**10.89**	**14.10**	**48.70**	**16.26**	**11.36**
Total P (ug/L)	**N:P (BULK)**	**Decline**	**52.08**	**0.0020**	**15.12**	**31.97**	**63.10**	**17.55**	**9.58**
Total P (ug/L)	**N:P OM-BULK**	**Decline**	**52.08**	**0.0048**	**17.03**	**55.85**	**125.08**	**-5.55**	**2.10**

Several other variables showed significant changes that corresponded to TP and TN (Table 1). Sedimentation variables (substrate embeddedness, mud-silt cover) both increased sharply at levels of TP and TN that also corresponded to significant water quality and biological changes (chlorophyll-a, periphyton C:P, filtrable residue). These sedimentation indicators were shown by Winemiller et al (2009) to correspond with increasing cover of pasture in the study watersheds, suggesting that pasture may be an important driver of both elevated nutrients and sediment problems in Ecoregion 29.

Variables that were indicators of submersed macrophyte cover (MCRPH_AB, MACRPHYT) and microalgae/biofilm cover (MICRALG) were expected to decline in response to TN and TP. However, they were too variable in their responses to be statistically significant. We expected these to decline because of their consistent response to TP in the study by King et al. (2009; Appendix B), which included an assessment of these variables in June 2008 at 26 of these 38 streams. In that event, macrophytes and biofilm thickness both significantly declined in response to TP levels > 20 ug/L. King et al. (2009) study used the 100-point transect method for estimating reach-scale cover of macrophytes, filamentous macroalgae, biofilm thickness, substrate, and sediment film thickness and found this approach to yield an excellent characterization of these variables. This current study used the TCEQ physical habitat assessment method, which was constrained to just 5 or 6 cross-sectional transects. Because of the high degree of spatial heterogeneity in the length of these reaches, we suggest that these transects are more likely to under or over estimate cover of these variables, and this may explain why these variables were not as effective as the field survey indicators of nutrient enrichment in the King et al. (2009) study.

Multivariate analysis of algal species composition among ecoregions

Ordination of sites based on the density of different algal species showed that periphyton communities growing on rocks (Cross Timbers) was clearly different than communities growing on sand/mud (Blackland Prairies, East Central Texas Plains; Figure 14-16). This was not unexpected. However, the ordination also revealed that algae growing on mud/silt in the Blackland Prairies was significantly different than East Central Texas Plains, with very little overlap (Figure 14; MRPP, $p<0.01$). This implies that ecoregional differences observed for fish (Winemiller et al. 2009) were also true for algae, thus analyses based on taxonomic composition will need to be stratified by ecoregion for both of these indicator groups.

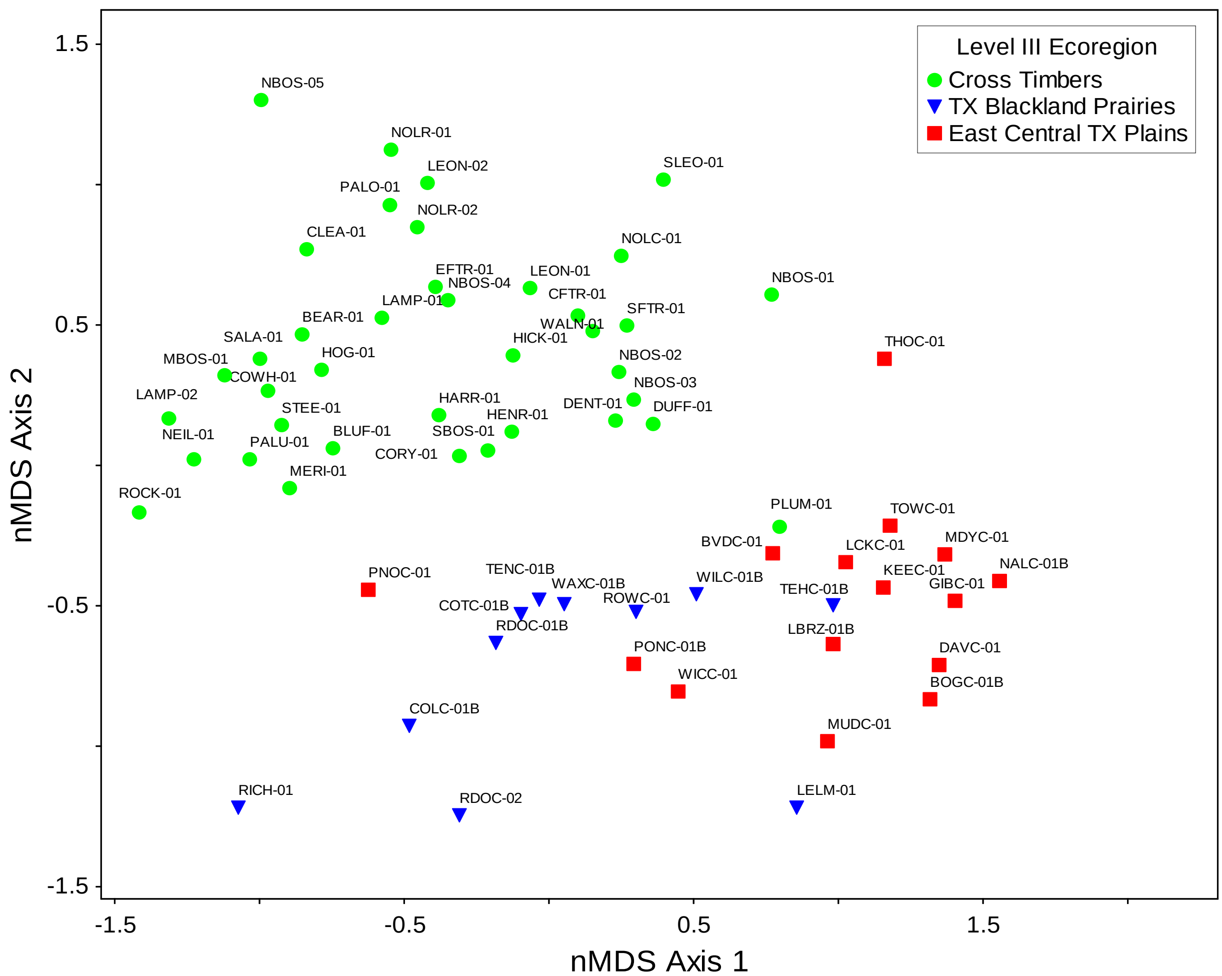

Figure 14. Nonmetric multidimensional scaling (nMDS) ordination of algal species composition among the three ecoregions.

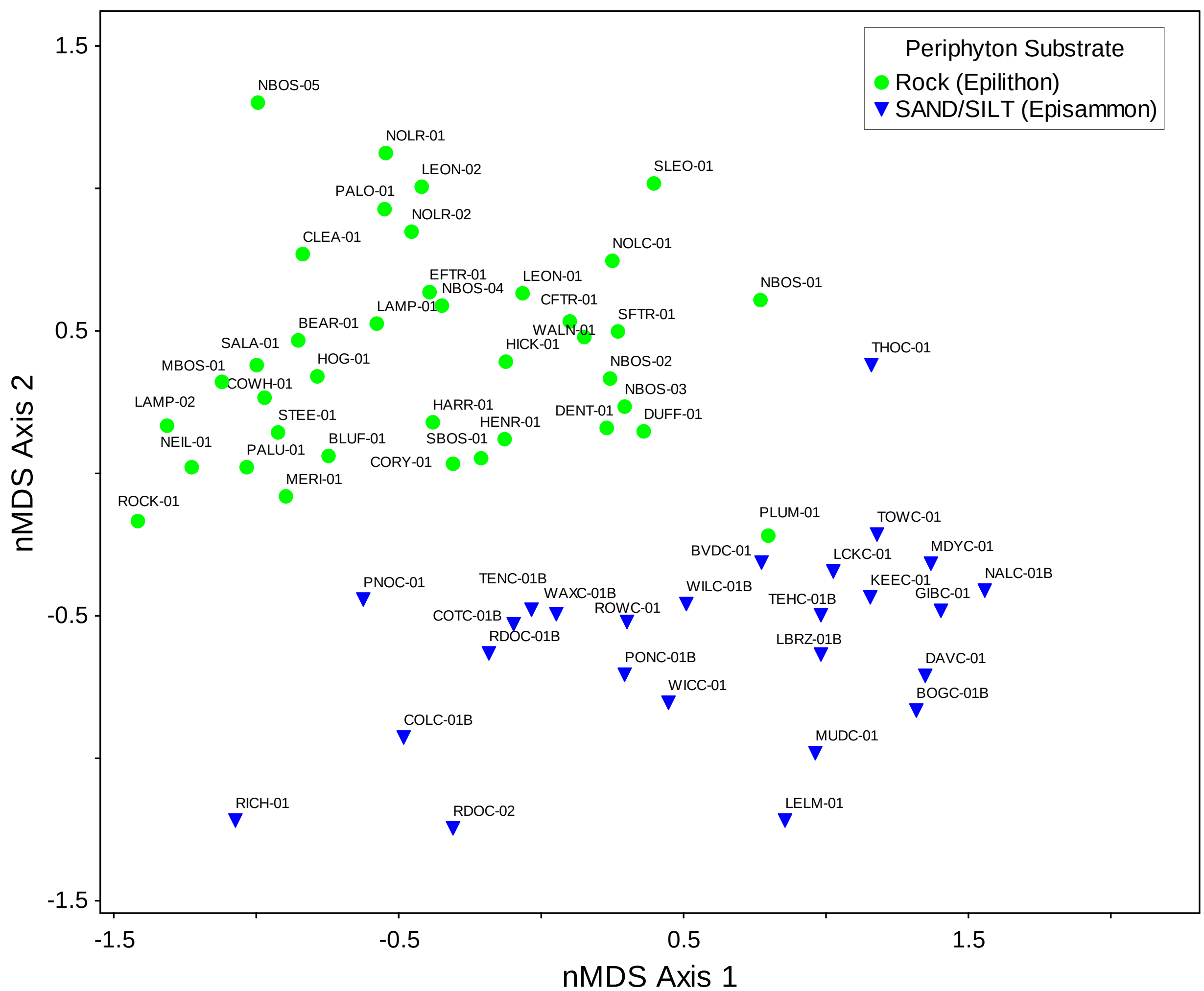

Figure 15. Nonmetric multidimensional scaling (nMDS) ordination of algal species composition between the two substrate types.

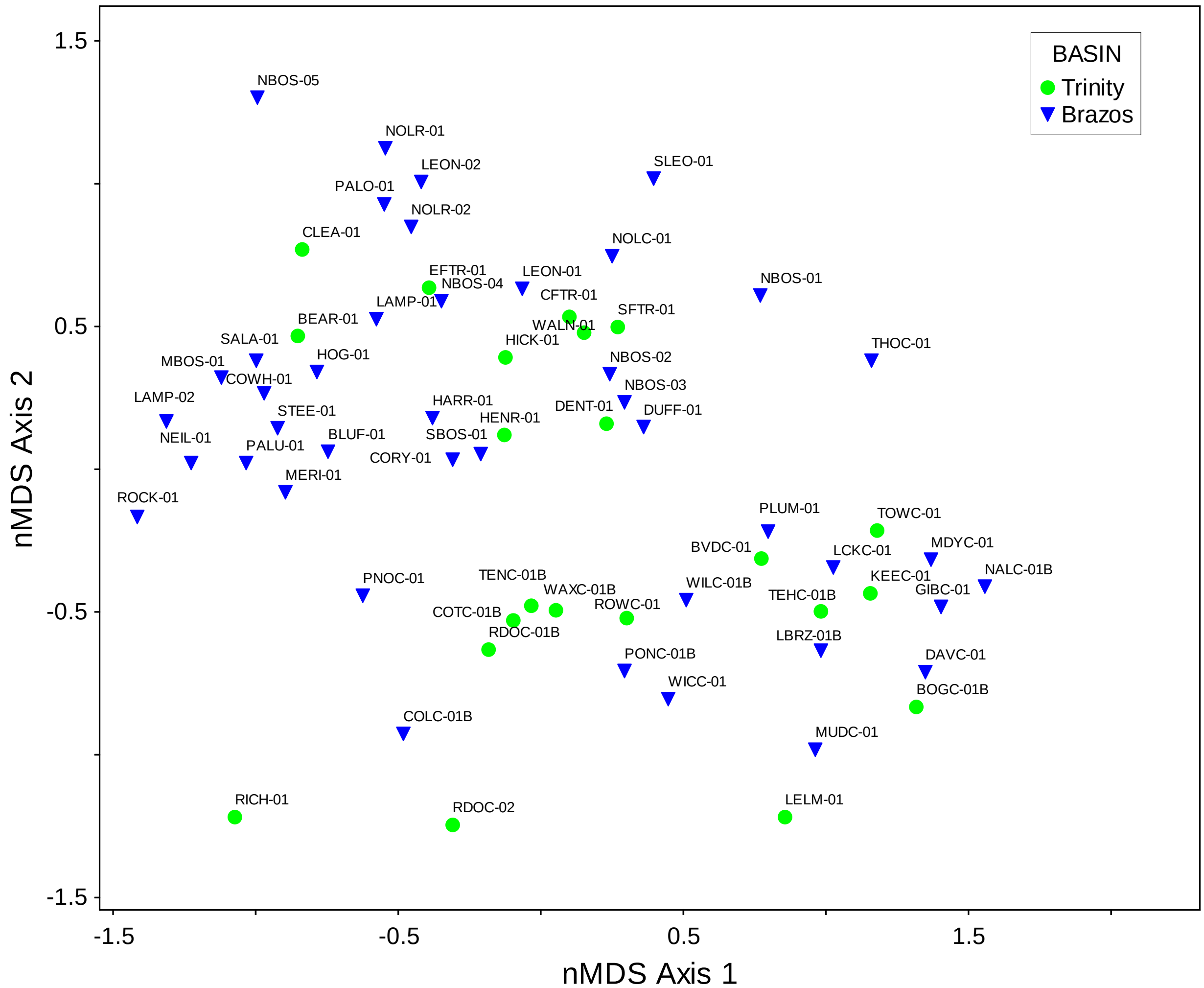

Figure 16. Nonmetric multidimensional scaling (nMDS) ordination of algal species composition among the two major river basins.

Algal species composition did not differ by the major river basins (Brazos and Trinity), however. Trinity sites were mostly enclosed within the cluster of Brazos sites in the ordination, or vice versa, regardless of substrate or ecoregion.This is important because it suggests that taxonomic composition metrics likely do not need to be stratified by basin for analyses or index development.

Ordination of algal species composition within Ecoregion 29 revealed a strong gradient along axis 1 that was highly correlated with numerous nutrient and nutrient-related environmental variables (Figures 17-19). Sites with low TP, TN, pasture, outfalls, sediment, and chloride and high periphyton C:N, C:P, and N:P ratios were grouped on the left side of the ordination, whereas sites with high values for these stressors were consistently grouped on the right side. This result is very similar to 2006 and 2007 data reported in King et al. (2009; Appendix B).

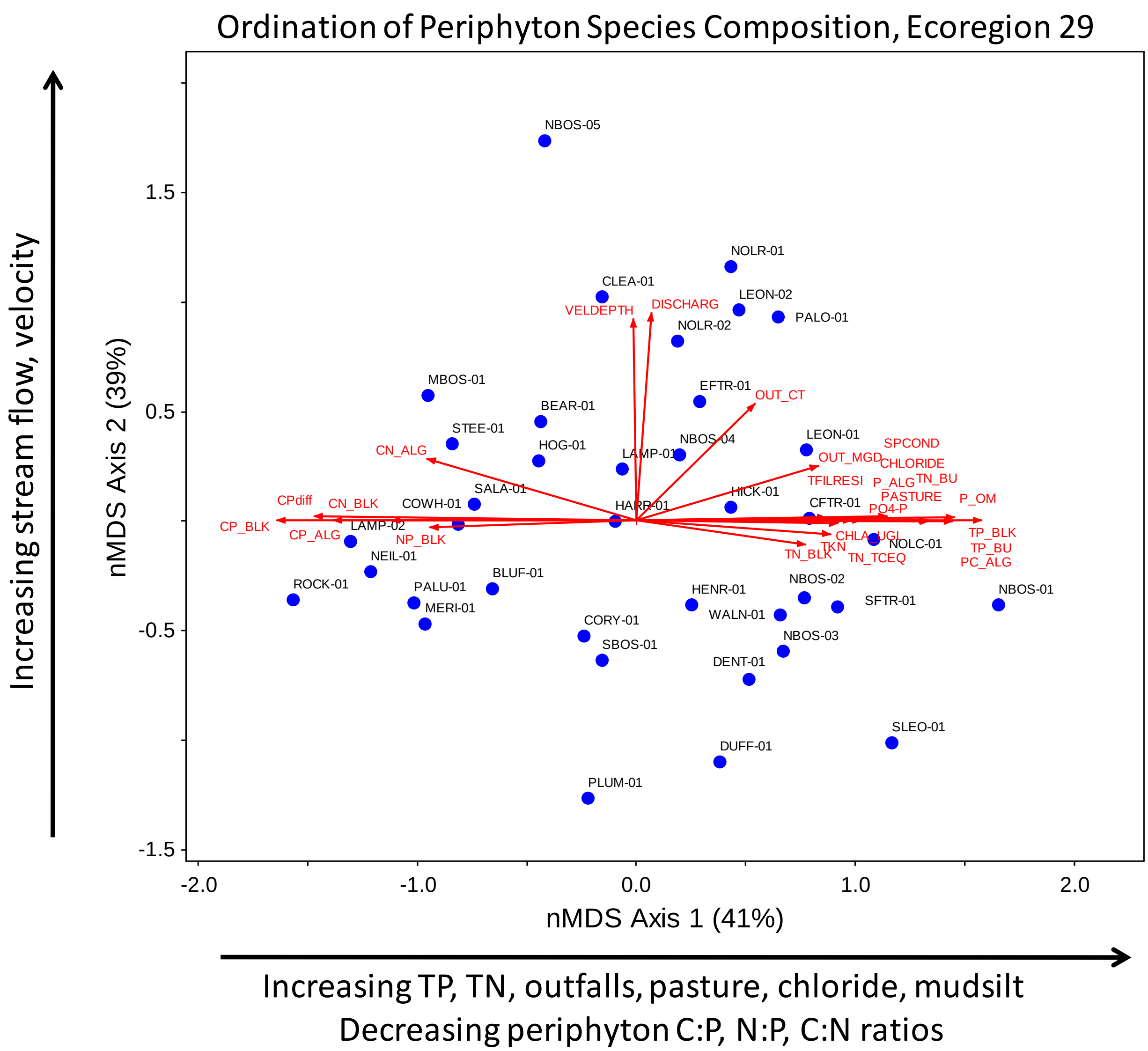

Figure 17. Nonmetric multidimensional scaling ordination of algal species composition among the 38 sites in Ecoregion 29 in summer 2008. Abundance data (no. of cells/cm^2) was $log_{10}(x+1)$ transformed prior to analysis. Bray-Curtis distance was used as the dissimilarity metric. Distances between sites in the ordination space are proportional to taxonomic dissimilarity (near=similar, far=dissimilar). In each figure, the red arrows (vectors) represent the direction and magnitude of significant ($p<0.05$) correlations between environmental variables and algal species composition. See Appendices A1-3 for full variable names.

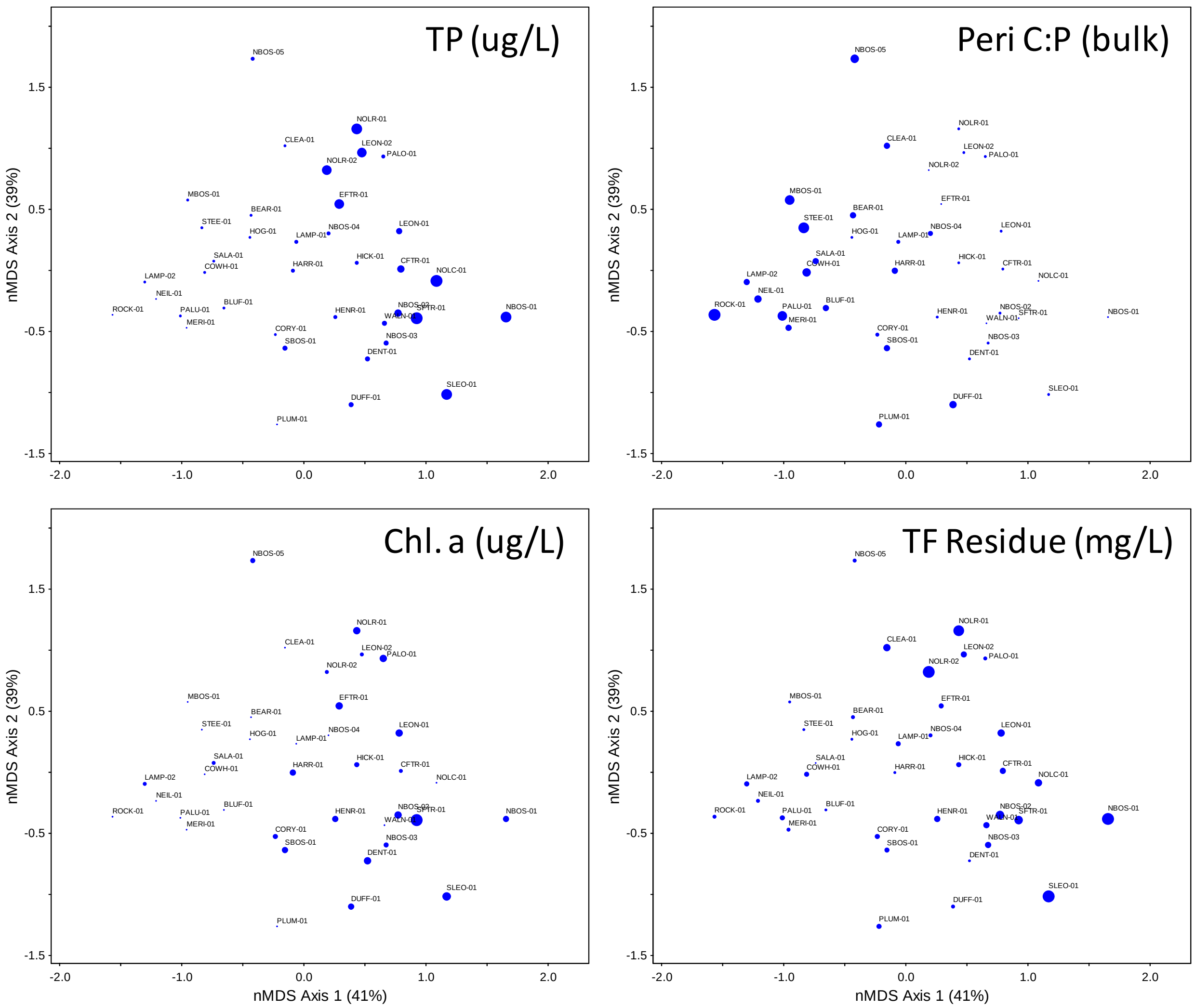

Figure 18. Nonmetric multidimensional scaling ordination of algal species composition among the 38 sites in Ecoregion 29 in summer 2008. The ordination diagram is identical to Figure 17, except that site symbols are scaled in proportion to measured values of surface-water TP, periphyton C:P (bulk), chlorophyll-a (water), and total filtrable residue (water).

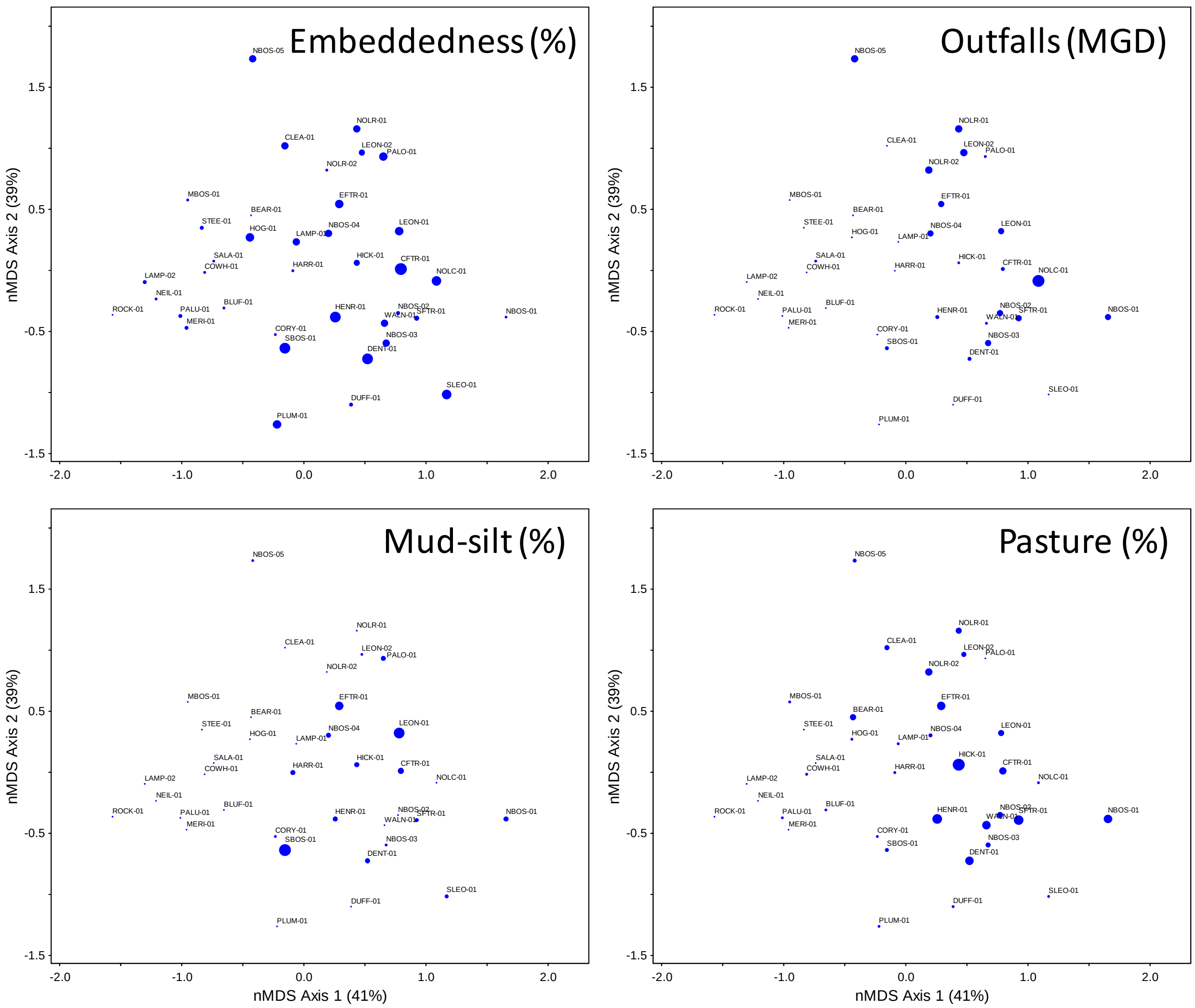

Figure 19. Nonmetric multidimensional scaling ordination of algal species composition among the 38 sites in Ecoregion 29 in summer 2008. The ordination diagram is identical to Figure 17, except that site symbols are scaled in proportion to measured values to substrate embeddedness, outfalls (permitted mgd in watershed), mud-silt cover (%), and pasture cover (% of watershed).

Algal species composition was not related to any nutrient or nutrient-related variable in Ecoregions 32 or 33 (Figure 20, 21). Even with the small sample sizes, algal taxonomic composition should have corresponded more closely to surface-water and periphyton chemistry than it did in these data sets. This implied that sand/mud periphyton samples were too variable to use reliably as nutrient indicators, and that alternative substrates (wood, artificial) should be considered for biological assessment in these soft-bottomed stream ecosystems.

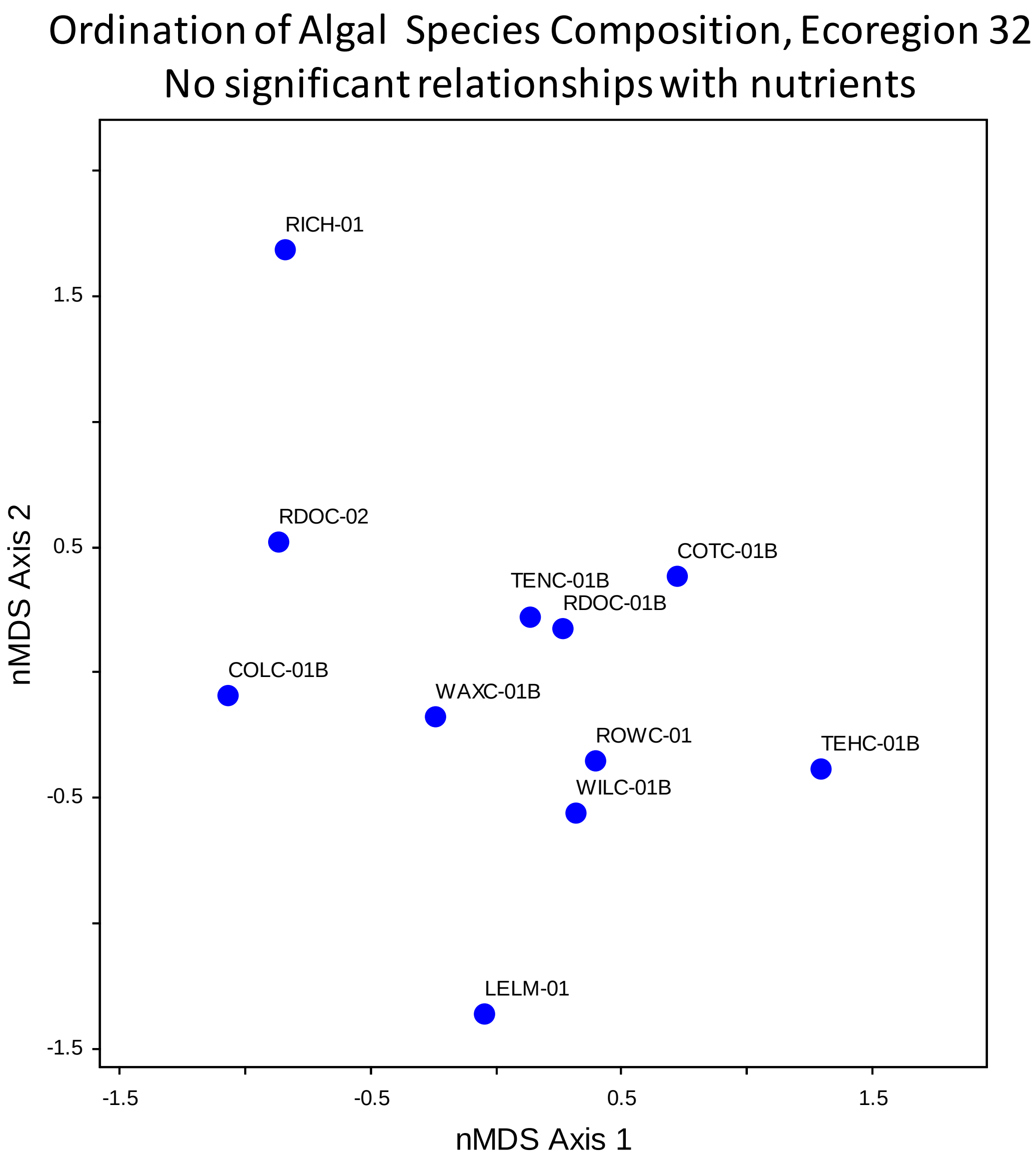

Figure 20. Nonmetric multidimensional scaling ordination of algal species composition among the 11 sites in Ecoregion 32 in summer 2008.

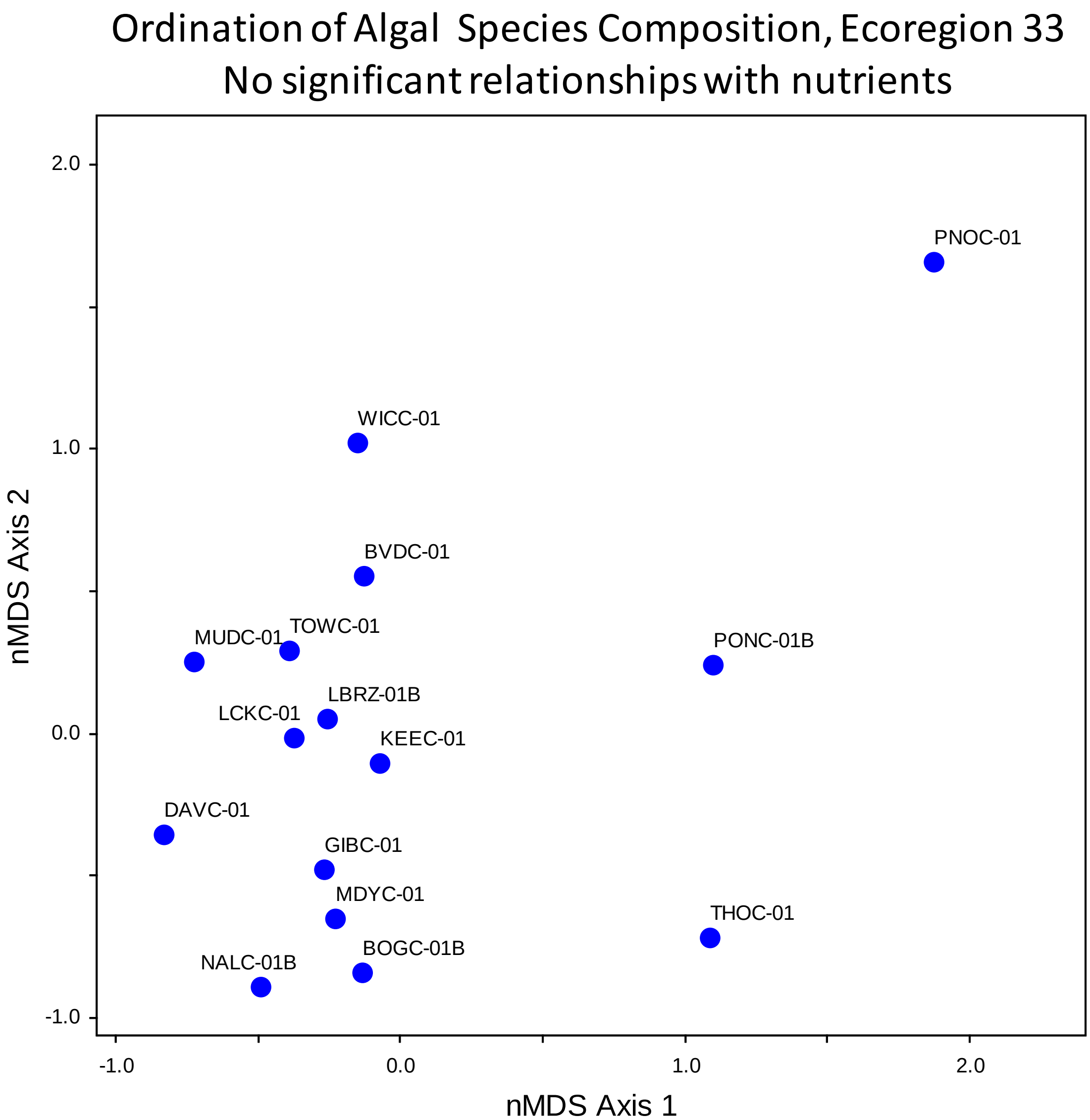

Figure 21. Nonmetric multidimensional scaling ordination of algal species composition among the 15 sites in Ecoregion 33 in summer 2008.

RESULTS AND INTERPRETATION, CONTINUED

Threshold responses of algal species to nutrient gradients in Ecoregion 29

Thirty-one algal species declined significantly in response to surface-water TP (Figure 22; Appendix A6). Most of these taxa declined between 15 and 25 ug/L TP. The TP level most likely to result in a community level decline (sum z-; Table 2) was 21 ug/L. Bootstrap confidence limit estimates suggested that this threshold may have been as low as 12 ug/L and highly likely to occur if TP exceeded 28 ug/L (Table 2).

TITAN also detected 36 algal species that proliferated rapidly with increasing TP in the wake of declines of other species (Figure 22; Appendix A6). Most of these species increased between 20 and 50 ug/L TP, but a few did not begin to appear until TP exceeded 500 ug/L (Figure 22). The community level threshold for increasing (positive responding) taxa was 40 ug/L TP (Table 2).

Fifteen and 28 taxa declined in response to TN and chloride, respectively (Figure 24; Appendix A6, Table 2). Some of these taxa differed from those that declined in response to TP, but the magnitude of the aggregate community response was lower than that of TP. Community-level threshold declines in algal species composition were most likely at 320 ug/L TN and 20 ug/L chloride (Table 2).

Most of the same taxa that declined in response to increasing TP declined in response to decreasing C:P ratios in the periphyton (Figure 25, Appendix A6). The consistency of this response is important because it demonstrates that changes in the amount of phosphorus in the periphyton itself results in sharp community changes that mirror the changes in response to surface-water TP. The level of C:P in the periphyton that led to the greatest overall decline in algal species was below 225 for OM samples and 335 for bulk periphyton.

The percentage cover of pasture in watersheds and the permitted volume of outfalls in watersheds (millions of gallons per day) both resulted in similar threshold declines in algal species as nutrients and nutrient related stressors (Figures 26, 27; Appendix A6). These variables also corresponded to sharp increases in taxa not found at sites with low levels of nutrients, sediment, and chloride (Figures 26, 27; Appendix A6). Watersheds exceeding 3.3% pasture cover and 0.31 MGD of permitted outfalls had the greatest overall declines in algal species, whereas pollution-indicator species proliferated in watersheds with > 7% pasture and >0.31 MGD of outfalls (Table 2).

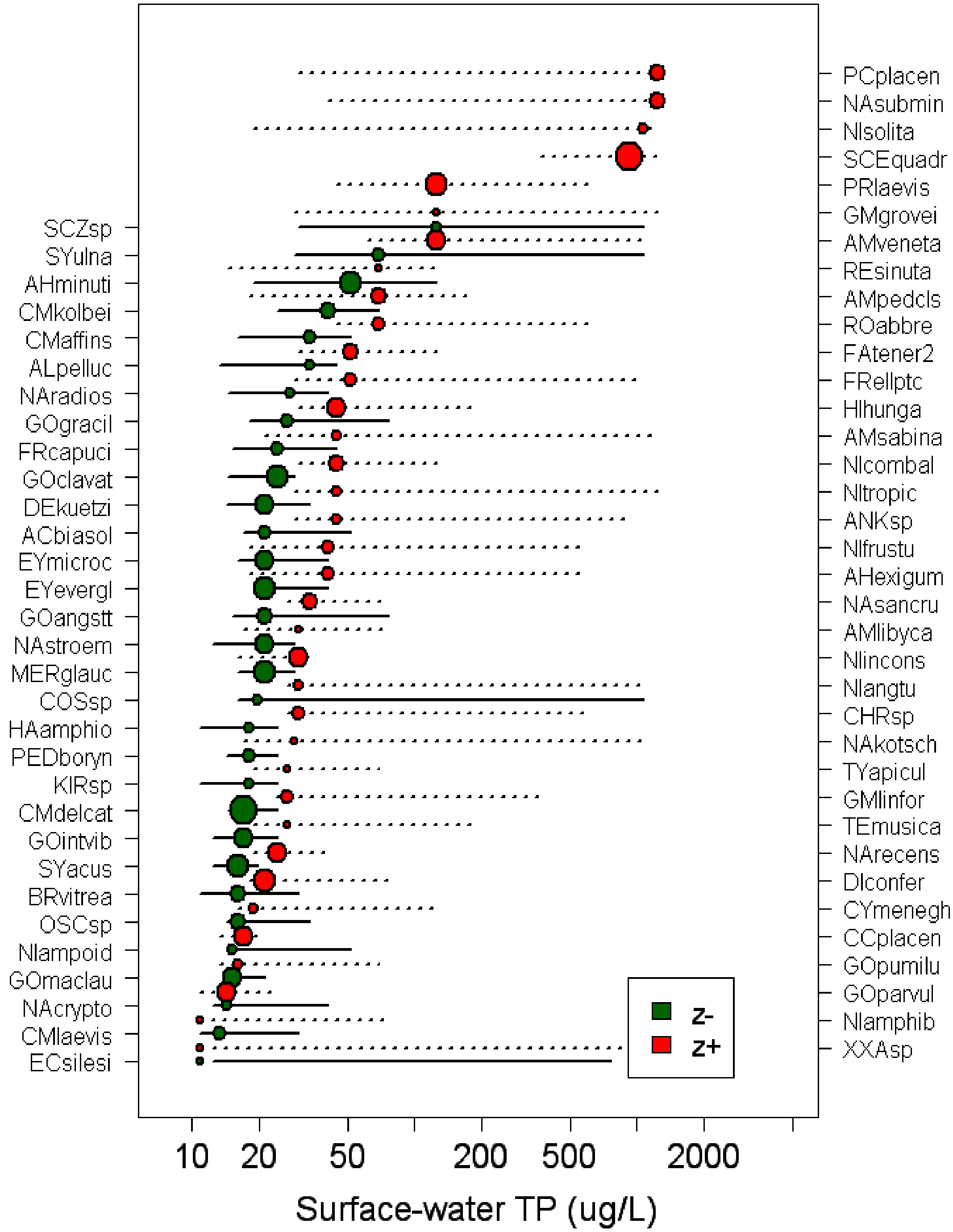

Figure 22. Results of Threshold Indicator Taxa ANalysis (TITAN) using surface-water TP a a predictor of threshold changes in individual algal species in Ecoregion 29 in summer 2008. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to TP. The observed TP threshold value (colored symbols) correspond to each taxon deemed to change significantly. Taxon IDs (see Appendix A5) are shown on the left (negative indicators) and right (positive indicators) y-axes, in rank order of their TP thresholds. Line segments around each symbol are 90% confidence intervals around the TP threshold. Symbol sizes correspond to the indicator score.

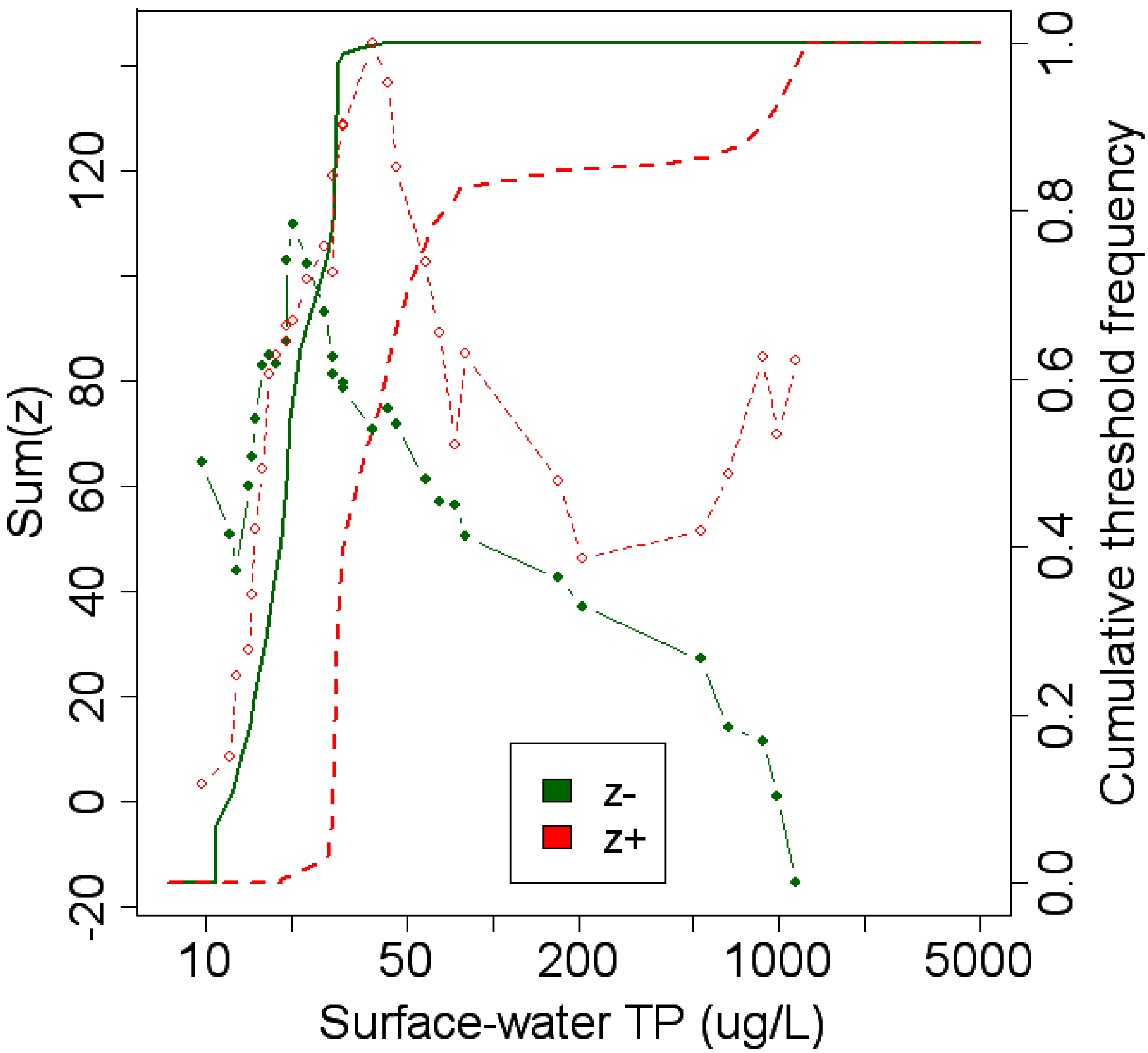

Figure 23. Results of Threshold Indicator Taxa ANalysis (TITAN) using surface-water TP a a predictor of threshold changes in community-level algae abundance data in Ecoregion 29 in summer 2008. Community responses are separated between the aggregate response of negative (sum(z-)) and positive (sum(z+)) threshold indicator taxa. The TP value resulting in the highest sum(z) value is the point in which the greatest cumulative negative (z-) or positive (z+) occurs. Bootstrapping is used to estimate the cumulative threshold frequency for negative (green) and positive (red) responses, respectively. See Table 2 for community level (sum(z)) thresholds.

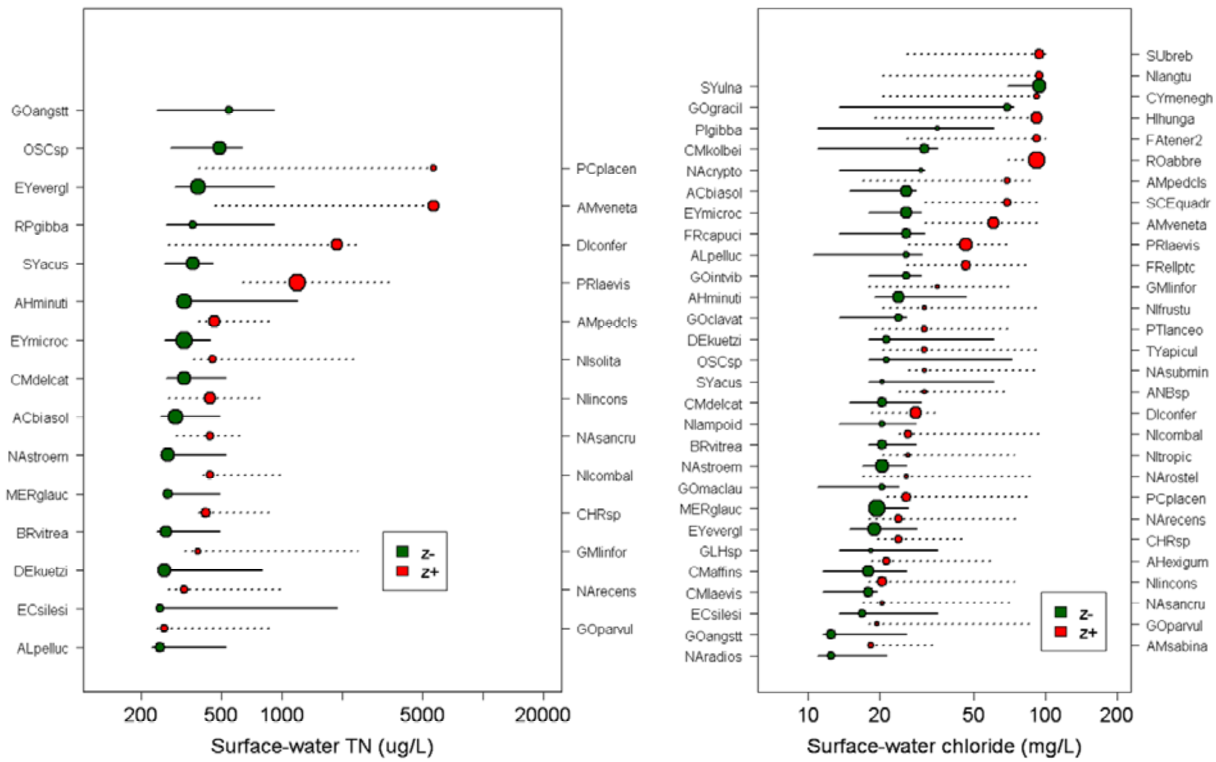

Figure 24. Results of Threshold Indicator Taxa ANalysis (TITAN) using surface-water TN (left panel) and chloride (right panel) as predictors of threshold changes in individual algal species in Ecoregion 29 in summer 2008. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to these predictors. The observed TN or chloride threshold value (colored symbols) correspond to each taxon deemed to change significantly, and the size of the symbol corresponds to the magnitude of the response. Taxon IDs (see Appendix A5) are shown on the left (negative indicators) and right (positive indicators) y-axes, in rank order of their thresholds. Line segments around each symbol are 90% confidence intervals around each observed threshold. Symbol sizes correspond to the indicator score.

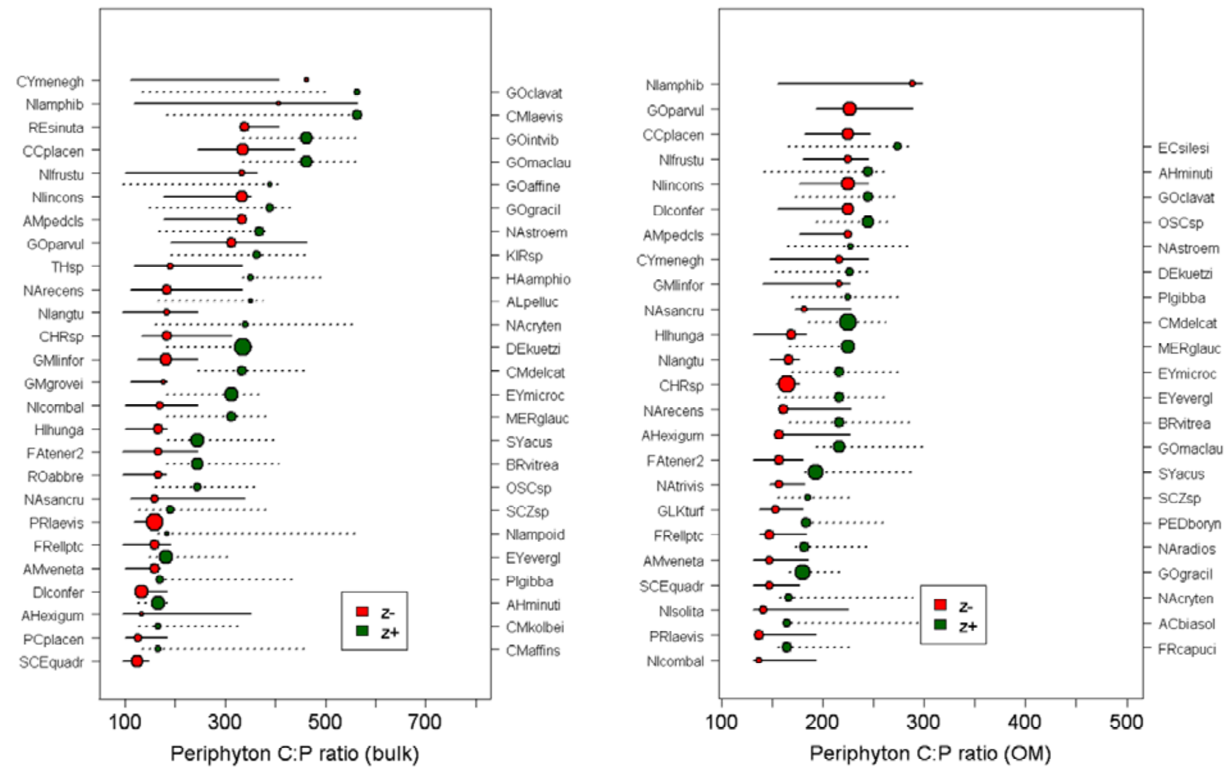

Figure 25. Results of Threshold Indicator Taxa ANalysis (TITAN) using periphyton C:P bulk (left panel) and periphyton C:P OM (right panel) as predictors of threshold changes in individual algal species in Ecoregion 29 in summer 2008. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to the C:P ratios in the periphyton. The observed C:P threshold value (colored symbols) correspond to each taxon deemed to change significantly, and the size of the symbol corresponds to the magnitude of the response. Taxon IDs (see Appendix A5) are shown on the left (negative indicators) and right (positive indicators) y-axes, in rank order of their C:P thresholds. Line segments around each symbol are 90% confidence intervals around the C:P threshold. Symbol sizes correspond to the indicator score.

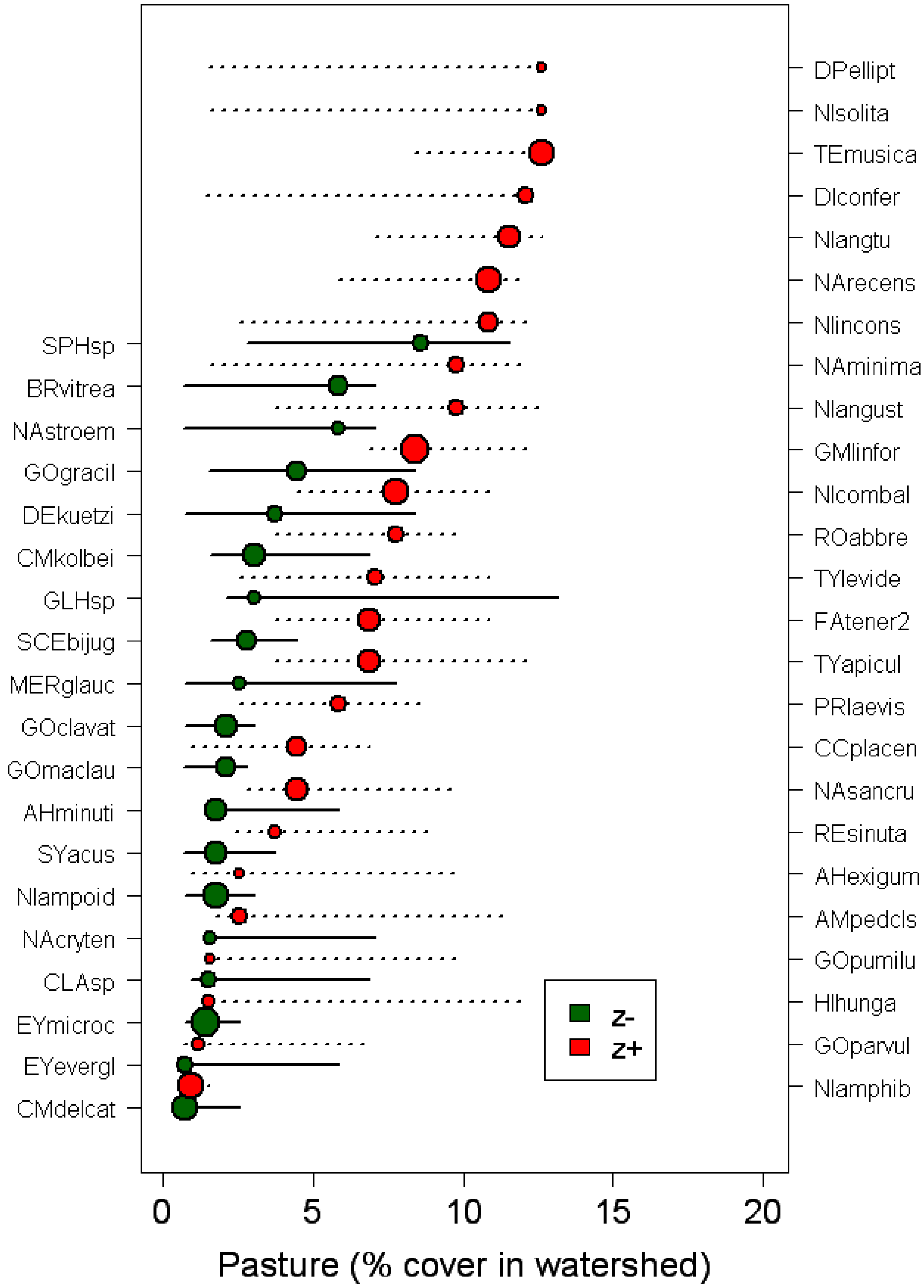

Figure 26. Results of Threshold Indicator Taxa ANalysis (TITAN) using % pasture cover in watersheds as a predictor of threshold changes in individual algal species in Ecoregion 29 in summer 2008. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to % pasture. The observed % pasture threshold value (colored symbols) correspond to each taxon deemed to change significantly. Taxon IDs (see Appendix A5) are shown on the left (negative indicators) and right (positive indicators) y-axes, in rank order of their % pasture thresholds. Line segments around each symbol are 90% confidence intervals around the % pasture threshold. Symbol sizes correspond to the indicator score.

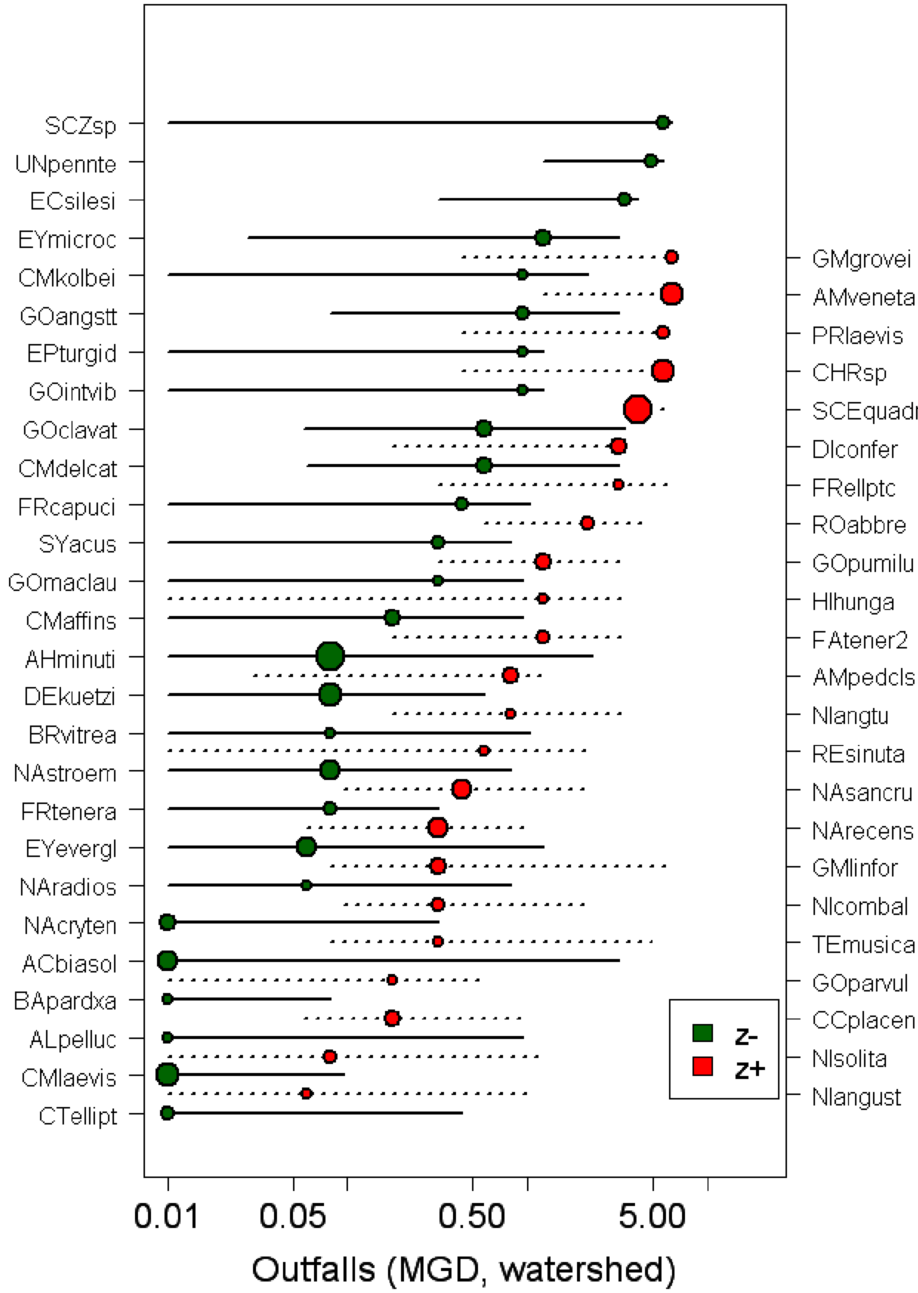

Figure 27. Results of Threshold Indicator Taxa ANalysis (TITAN) using outfalls (mgd) in watersheds as a predictor of threshold changes in individual algal species in Ecoregion 29 in summer 2008. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to outfalls. The observed outfall (mgd) threshold value (colored symbols) correspond to each taxon deemed to change significantly. Taxon IDs (see Appendix A5) are shown on the left (negative indicators) and right (positive indicators) y-axes, in rank order of their outfall thresholds. Line segments around each symbol are 90% confidence intervals around the outfall threshold. Symbol sizes correspond to the indicator score.

Table 2. Community-level results from Threshold Indicator Taxa Analysis (TITAN) on **algal species** composition from **Ecoregion 29** in response to water and periphyton nutrient concentrations, sedimentation, outfalls, pasture, and chloride. Thresholds (*Obs.*) are based on the value of the predictor resulting in the greatest aggregate decrease (sum(z-)) or increase (sum(z+)) in the frequency and abundance of taxa in the community. Taxa responses associated with lower nutrient or stressor conditions are shown in **bold.** The lower (*10%*), middle (*50%*), and upper (*90%*) quantiles of 1,000 bootstraps represent measures of uncertainty around the observed threshold.**Note that lower C:P values = higher P enrichment relative to organic carbon in the periphyton, thus taxa that "decrease" sharply in response to increasing C:P are associated with higher levels of P-enrichment,, whereas "increaser" taxa are associated with lower levels of P enrichment.*. See previous figures for details.

				Bootstrap Threshold Quantiles		
	Threshold Indicator	Taxa response > threshold	Obs. threshold	10%	50%	90%
TP (ug/L)	sumz-	**Decline**	**21.43**	**12.44**	**19.68**	**28.95**
	sumz+	Increase	40.73	28.95	40.73	932.17
TN (ug/L)	sumz-	**Decline**	**384.67**	**225.33**	**271.00**	**462.50**
	sumz+	Increase	440.83	402.50	462.50	5723.33
Periphyton C:P (OM)	sumz-	Decline	216.60	141.57	182.03	216.60
	sumz+	**Increase**	**225.08**	**182.03**	**227.57**	**295.89**
Periphyton C:P (bulk)	sumz-	Decline	159.00	95.50	165.95	245.12
	sumz+	**Increase**	**335.81**	**178.01**	**191.55**	**438.57**
Pasture (%)	sumz-	**Decline**	**3.26**	**0.77**	**2.42**	**3.26**
	sumz+	Increase	7.05	3.26	8.61	12.36
Outfalls (MGD)	sumz-	**Decline**	**0.32**	**0.01**	**0.18**	**0.58**
	sumz+	Increase	0.32	0.18	0.58	5.69
Mud-silt (%)	sumz-	**Decline**	**0.00**	**0.00**	**1.21**	**3.75**
	sumz+	Increase	15.42	2.20	13.75	21.17
Chloride (mg/L)	sumz-	**Decline**	**20.50**	**18.00**	**20.50**	**26.00**
	sumz+	Increase	31.00	24.00	30.00	92.50

RESULTS AND INTERPRETATION, CONTINUED

Multivariate analysis of fish species composition among ecoregions

Winemiller et al (2009) thoroughly describe the relationships between fish communities, habitat variables, and watershed physiographic variables among ecoregions using the summer 2008 data set. Therefore, results presented here are limited to nutrient and nutrient-related variables not included in that report.

Fish community structure in Ecoregion 29 was strongly related to many nutrient and nutrient-related variables (Figure 28). Sites with low TP, chloride, substrate embeddedness, mud-silt, chlorophyll-a, filtrable and nonfiltrable residue and high periphyton C:P, C:N, and N:P ratios were grouped on the left end of axis 1, the most important axis of community structure (Figures 28-30). Watershed outfalls and pasture also were significantly related to fish communities along this axis and suggested that both were potential drivers of these biological changes. These local and watershed variables were therefore selected as predictors of potential threshold changes in fish species and subsequent metrics based on combinations of fish species (see *Threshold responses of fish species to nutrient gradients in Ecoregion 29*, next section).

Fish communities in Ecoregion 32 and 33 were weakly related to a few nutrient or nutrient-related variables (Figures 31, 32). Because of small sample sizes, insufficient sites with low levels of nutrients, and outliers, none of these relationships was sufficiently strong to be reliable or interpretable. However, these weak trends imply that watershed stressors such as outfalls, rowcrop, pasture, and impervious cover are likely influencing fish communities in these ecoregions, and that nutrient enrichment and sedimentation probably play a role in reduction of biological integrity in these stream ecosystems. Additional sites that fill gaps in the spatial distribution of sites in these ecoregions are needed to adequately evaluate biological responses to habitat, water quality, and watershed sources of abiotic stressors.

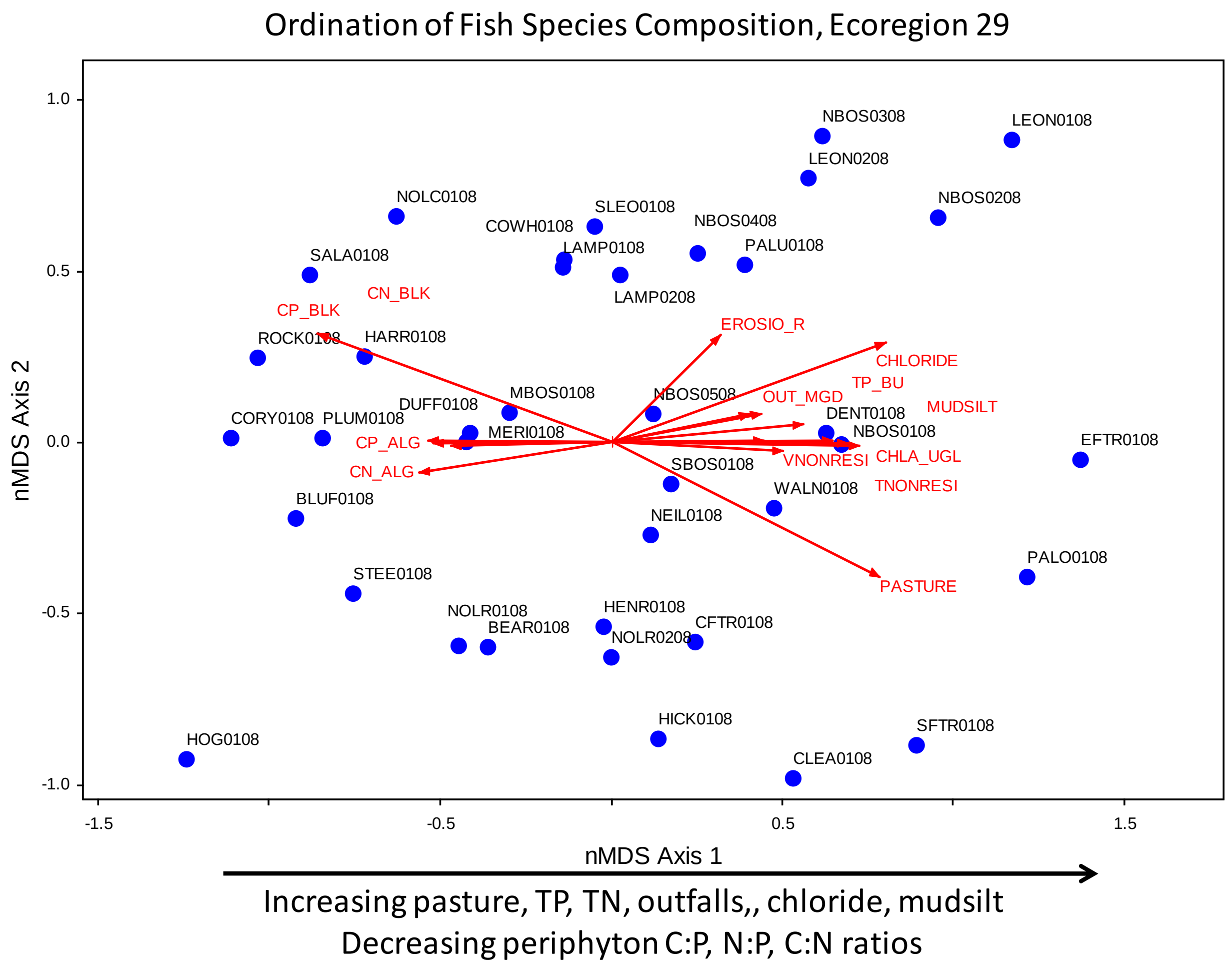

Figure 28. Nonmetric multidimensional scaling ordination of fish species composition among the 38 sites in Ecoregion 29 in summer 2008. Abundance data was $\log_{10}(x+1)$ transformed prior to analysis. Bray-Curtis distance was used as the dissimilarity metric. Distances between sites in the ordination space are proportional to taxonomic dissimilarity (near=similar, far=dissimilar). In each figure, the red arrows (vectors) represent the direction and magnitude of significant ($p<0.05$) correlations between environmental variables and fish species composition. See Appendices for full variable names.

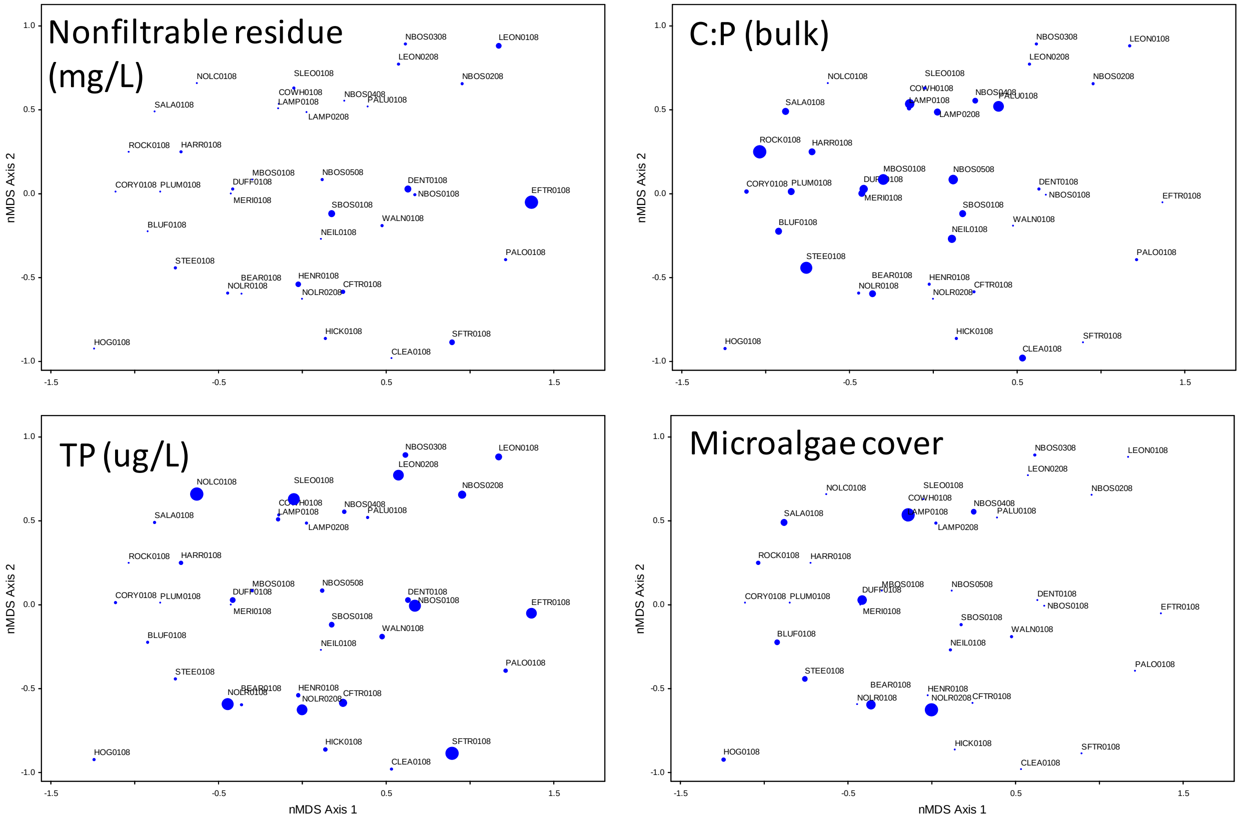

Figure 29. Nonmetric multidimensional scaling ordination of fish species composition among the 38 sites in Ecoregion 29 in summer 2008. The ordination diagram is identical to Figure 28, except that site symbols are scaled in proportion to measured values of surface-water TP, periphyton C:P (bulk), microalgae cover, and total nonfiltrable residue (water).

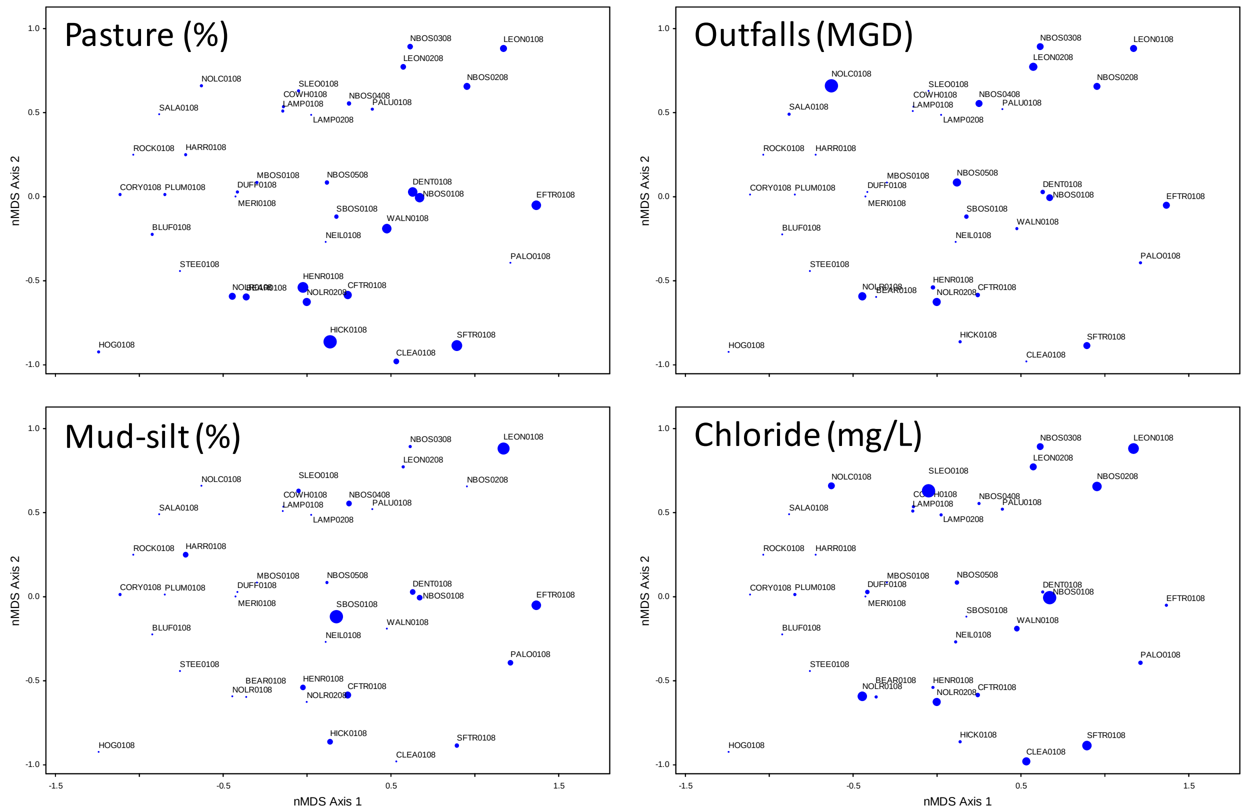

Figure 30. Nonmetric multidimensional scaling ordination of fish species composition among the 38 sites in Ecoregion 29 in summer 2008. The ordination diagram is identical to Figure 28, except that site symbols are scaled in proportion to measured values of pasture (%), outfalls (mgd), mud-silt (%), and chloride (mg/L).

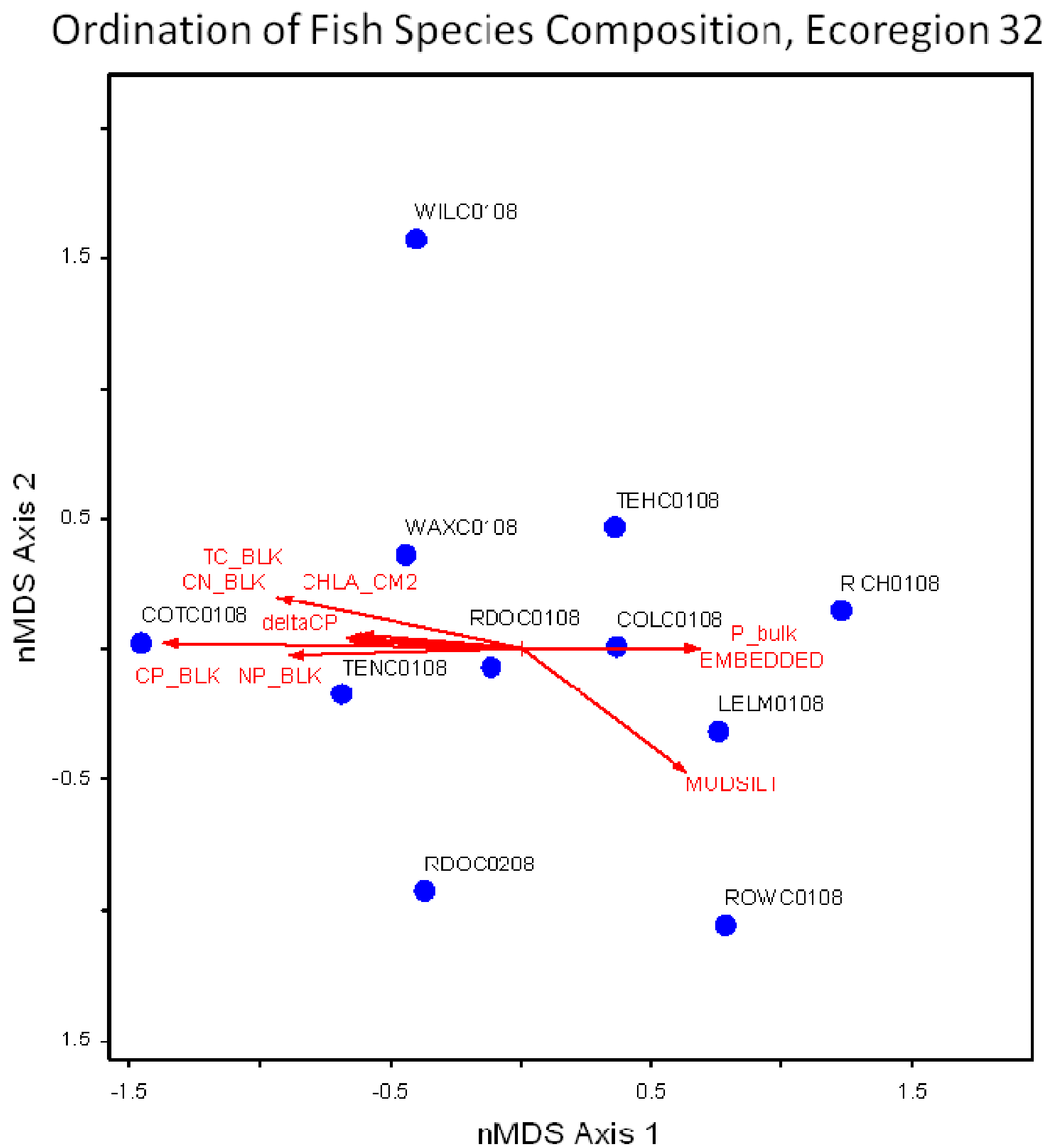

Figure 31. Nonmetric multidimensional scaling ordination of fish species composition among the 11 sites in Ecoregion 32 in summer 2008. Abundance data was $\log_{10}(x+1)$ transformed prior to analysis. Bray-Curtis distance was used as the dissimilarity metric. Distances between sites in the ordination space are proportional to taxonomic dissimilarity (near=similar, far=dissimilar). In each figure, the red arrows (vectors) represent the direction and magnitude of significant ($p<0.05$) correlations between environmental variables and fish species composition. See Appendices for full variable names.

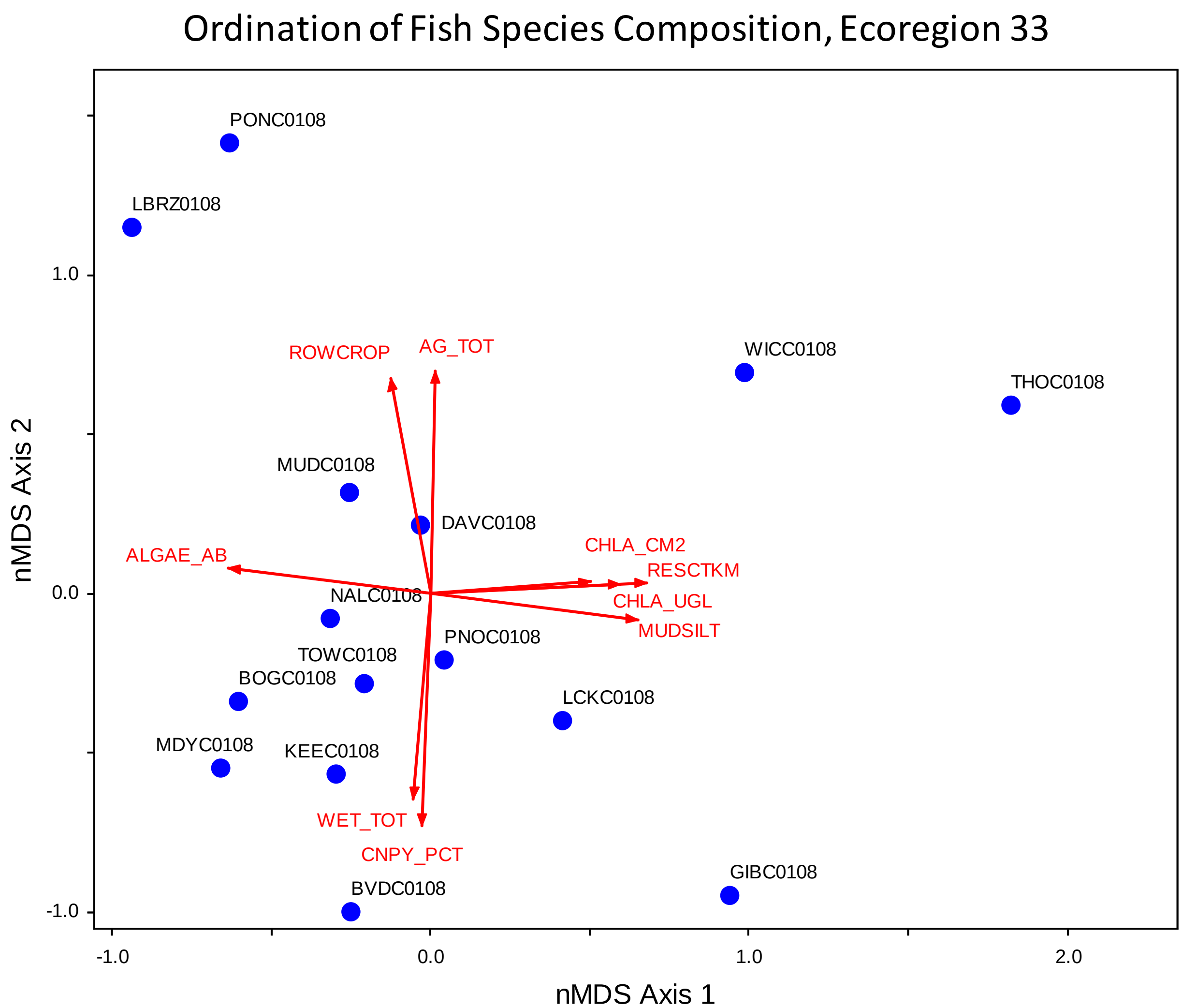

Figure 32. Nonmetric multidimensional scaling ordination of fish species composition among the 15 sites in Ecoregion 33 in summer 2008. Abundance data was $\log_{10}(x+1)$ transformed prior to analysis. Bray-Curtis distance was used as the dissimilarity metric. Distances between sites in the ordination space are proportional to taxonomic dissimilarity (near=similar, far=dissimilar). In each figure, the red arrows (vectors) represent the direction and magnitude of significant ($p<0.05$) correlations between environmental variables and fish species composition. See Appendices for full variable names.

RESULTS AND INTERPRETATION, CONTINUED

Threshold responses of fish species to nutrient gradients in Ecoregion 29

TITAN revealed that four fish species significantly declined in response to surface-water TP (Figure 33; Appendix A7). Three of these species (CYPRVENU=*Cyprinella venusta,* blacktail shiner; ETHESPEC=*Etheostoma spectabile*, orangethroat darter; CAMPANOM=*Campostoma anomalum,* central stoneroller) had observed thresholds between 15 and 25 ug/L TP. The TP level most likely to result in a community level decline (sum z-) was 28 ug/L (Table 3).

Four fish species sharply increased in abundance and frequency of occurrence in sites with elevated TP: CYPRLUTR (*Cyprinella lutrensis,* red shiner), PIMEVIGI (*Pimephales vigilax*, bullhead minnow), LEPIOSSE (*Lepisosteus osseus*, longnose gar), and CARPCARP (*Carpiodes carpio*, river carpsucker) (Figure 33; Appendix A7). The community-level threshold for taxa that proliferated with TP enrichment was 30 ug/L (Table 3).

Most of these same species either declined or increased in response to periphyton C:P ratios, chloride, mud-silt cover, substrate embeddness, outfalls, and pasture (Table 3; Figure 34). Additional fish species that significantly declined in response to one or more of these stressors included *Fundulus notatus* (FUNDNOTA), *Lepomis gulosus* (LEPOGULO), *Notropis volucellus* (NOTRVOLU), *Moxostoma congestum* (MOXOCONG), and *Lepomis cyanellus* (LEPOCYAN) (Figure 34, Appendix A7).

Additional species that proliferated with increasing levels of nutrients or nutrient-related stressors included *Cyprinus carpio* (CYPRCARP), *Lythurus umbratilis* (LYTHUMBR), *Dorosoma cepedianum* (DOROCEPE), *Pylodictis olivaris* (PYLOOLIV), and *Pomoxis annularis* (POMOANNU) (Figure 34; Appendix A7). Most of these species are typically associated with turbid low gradient streams or reservoirs.

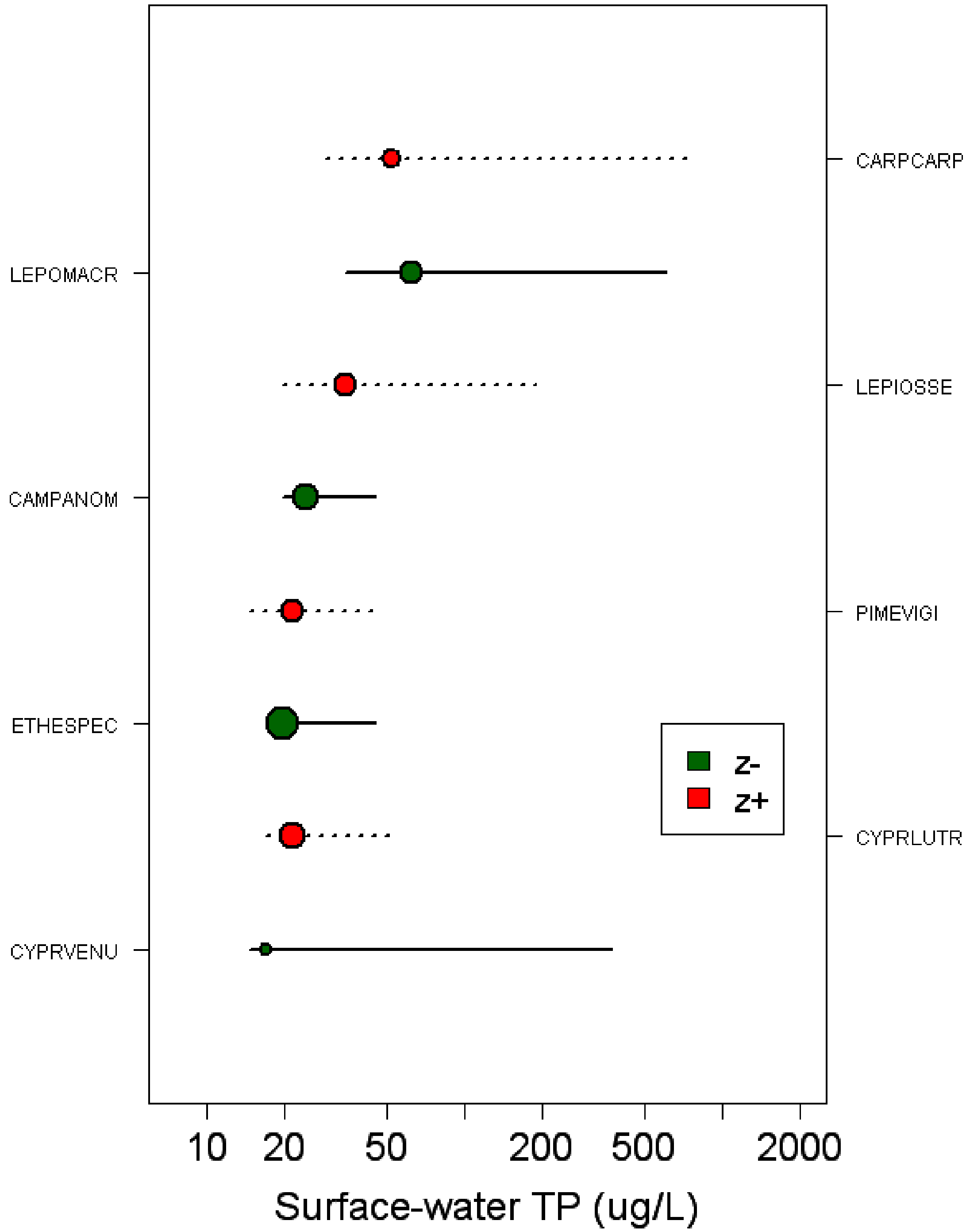

Figure 33. Results of Threshold Indicator Taxa ANalysis (TITAN) using surface-water TP as a predictor of threshold changes in individual fish species distributions in Ecoregion 29 in summer 2008.

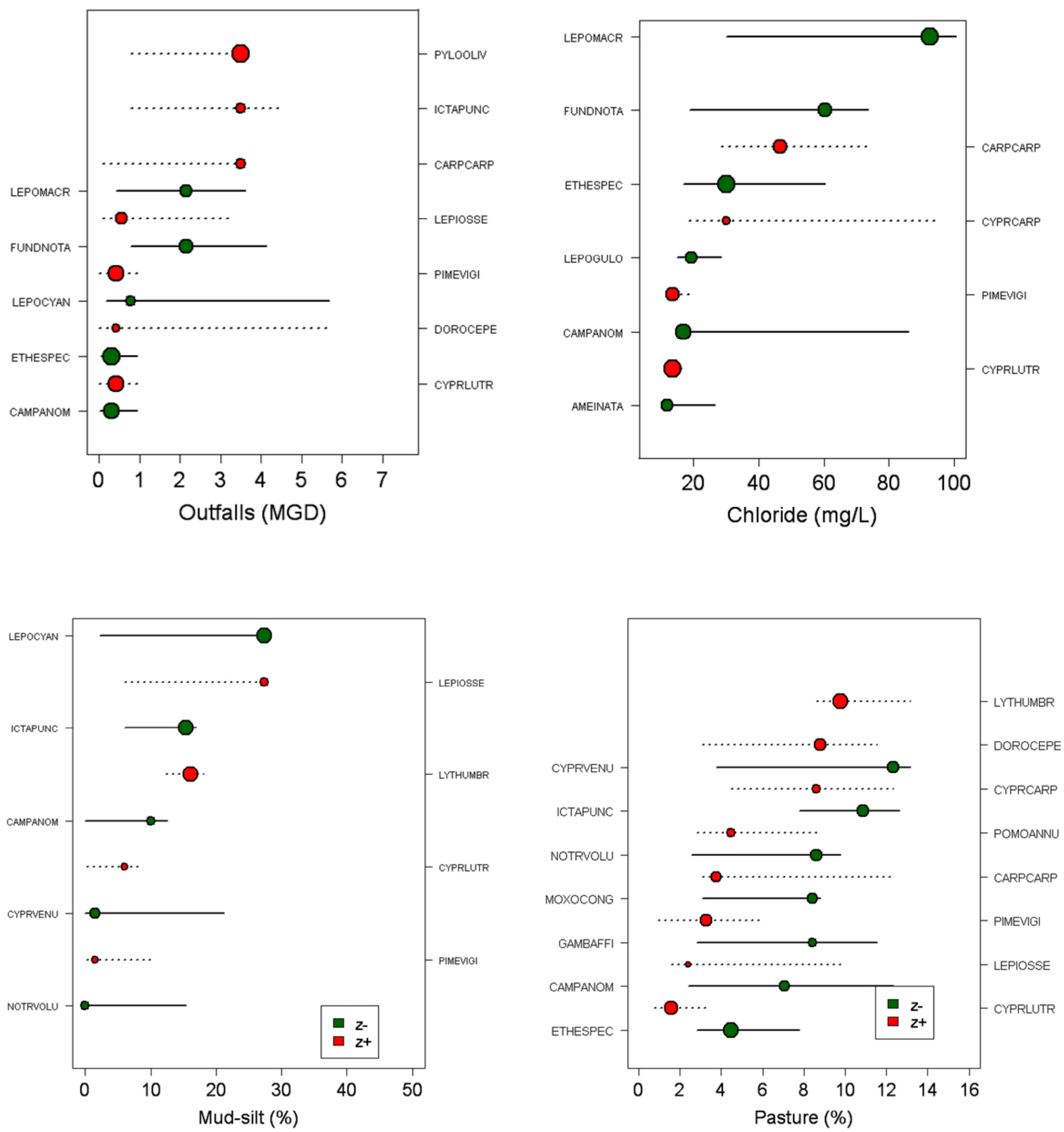

Figure 34. Results of Threshold Indicator Taxa ANalysis (TITAN) using outfalls, chloride, mud-silt cover, and pasture as predictors of threshold changes in individual fish species distributions in Ecoregion 29 in summer 2008.

Table 3 Community-level results from Threshold Indicator Taxa Analysis (TITAN) on **fish** species composition from **Ecoregion 29** in response to TP, sedimentation, outfalls, pasture, and chloride. Thresholds (*Obs.*) are based on the value of the predictor resulting in the greatest aggregate decrease (sum(z-)) or increase (sum(z+)) in the frequency and abundance of taxa in the community. Taxa responses associated with lower nutrient or stressor conditions are shown in **bold.** The lower (*10%*), middle (*50%*), and upper (*90%)* quantiles of 1,000 bootstraps represent measures of uncertainty around the observed threshold.

				Bootstrap Threshold Quantiles		
Predictor	Threshold Indicator	Taxa response > threshold	Obs. threshold	10%	50%	90%
TP (ug/L)	**sumz-**	**Decline**	**27.77**	**17.03**	**30.18**	**81.84**
	sumz+	Increase	30.18	24.22	34.18	69.78
Mud-silt (%)	**sumz-**	**Decline**	**0.92**	**0.00**	**7.25**	**16.04**
	sumz+	Increase	15.00	6.00	16.04	21.17
Outfalls (MGD)	**sumz-**	**Decline**	**2.16**	**0.02**	**0.80**	**3.25**
	sumz+	Increase	0.31	0.09	0.80	3.27
Pasture (%)	**sumz-**	**Decline**	**8.61**	**3.26**	**7.05**	**9.79**
	sumz+	Increase	3.26	2.81	7.05	12.07
Chloride (mg/L)	**sumz-**	**Decline**	**18.50**	**12.50**	**21.50**	**60.50**
	sumz+	Increase	35.00	13.50	24.00	72.50

Based on these and results described in Winemiller et al. (2009), we evaluated five univariate variables as potential new fish metrics of nutrient or nutrient-related problems in streams of Ecoregion 29:

- Fish community index (nMDS Axis 1). Site values are scores along the primary axis of variation in fish community structure from non-metric multidimensional scaling ordination of the 38 sites in Ecoregion 29 during summer 2008. Low (negative) scores represent sites that are most dissimilar from sites with high levels of outfalls, pasture, nutrients, chloride, and sediment (high, positive scores on axis 1).
- Percent abundance of the key grazing herbivore (*Campostoma anomalum*, central stoneroller). *Campostoma* was found to decline significantly in response to pasture, outfalls, embeddedness, and mud-silt in Winemiller et al. (2009), and additionally to chloride, TP, and C:P periphyton in this study. *Campostoma* plays a fundamental role in stream ecosystem processes in these streams by grazing on periphyton, recycling nutrients, exporting sediment, and as a primary food resource for native predator fishes such as spotted bass (*Micropterus punctalatus*).
- Percent abundance of darters (*Etheostoma*). *Etheostoma spectabile* was the dominant benthic invertivore in clear-water, low nutrient streams in Ecoregion 29, but rapidly declined with increasing nutrient enrichment, sedimentation, chloride, and drivers of these stressors (outfalls, pasture). Other related species (*Percina* spp.) were too infrequently collected to determine statistical significance but likely were negatively affected by these stressors as well. Primarily riffle, crevice-dwelling fish, these fish are mechanistically linked to benthic processes in streams and are another key indicator of biological integrity in these ecosystems.
- Percent abundance of nutrient-intolerant cyprinids (*Cyprinella venusta, Notropis volucellus*). Blacktail shiners are common in most streams in Ecoregion 29, but their percent contribution to community structure clearly declined as nutrient enrichment and sedimentation increased. Mimic shiner was also sensitive to these stressors. Note that classification of "intolerant" here is independent of TCEQ or other tolerant/intolerant classifications.

- Percent abundance of nutrient-tolerant cyprinids (*Cyprinella lutrensis, Pimephales vigilax*). Both of these species showed sharp increases in abundance with nutrient enrichment, as indicated by TITAN. Although these are native species and contribute positively to "number of native cyprinids", a metric used in the TCEQ IBI, these species are in fact very tolerant of pollution and benefit from human alterations to streams. Red shiners have been shown through historical analysis of Brazos River seine data (T. Bonner, unpublished data) to have markedly increased in abundance in the past 30-50 years while other native cyprinds have declined, a phenomenon coincident with dam construction and water quality declines in the mainstem Brazos. *Pimephales vigilax*, or bullhead minnow, is a close relative to the toxicological test organism *Pimephales promelas*, or fathead minnow, used because of its ease in reproduction and hardiness. Note that classification of "tolerant" here is independent of TCEQ or other tolerant/intolerant classifications.

Some of the other species found to be negative or positive threshold indicators by TITAN may also serve as stressor-specific metrics of biological integrity, but these responses need further evaluation.

Figures 35-39 and Table 4 illustrate that indeed these univariate metrics all significantly showed threshold responses to all of the stressors identified in the ordination and TITAN analyses.

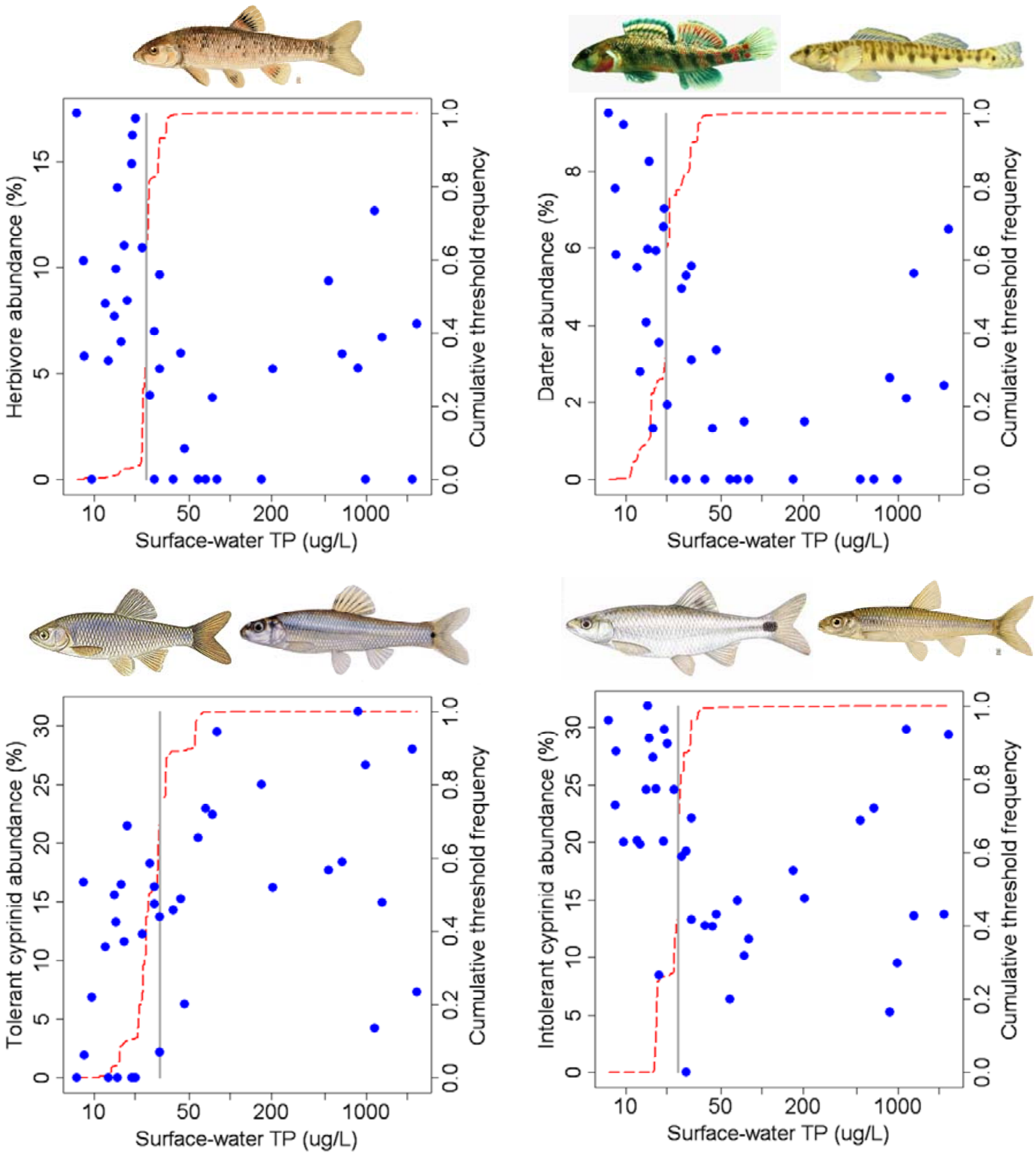

Figure 35. Results on nonparametric changepoint analysis using surface water TP as a predictor of threshold responses in the four proposed new fish metrics of nutrient-related reduction in biological integrity in Ecoregion 29.

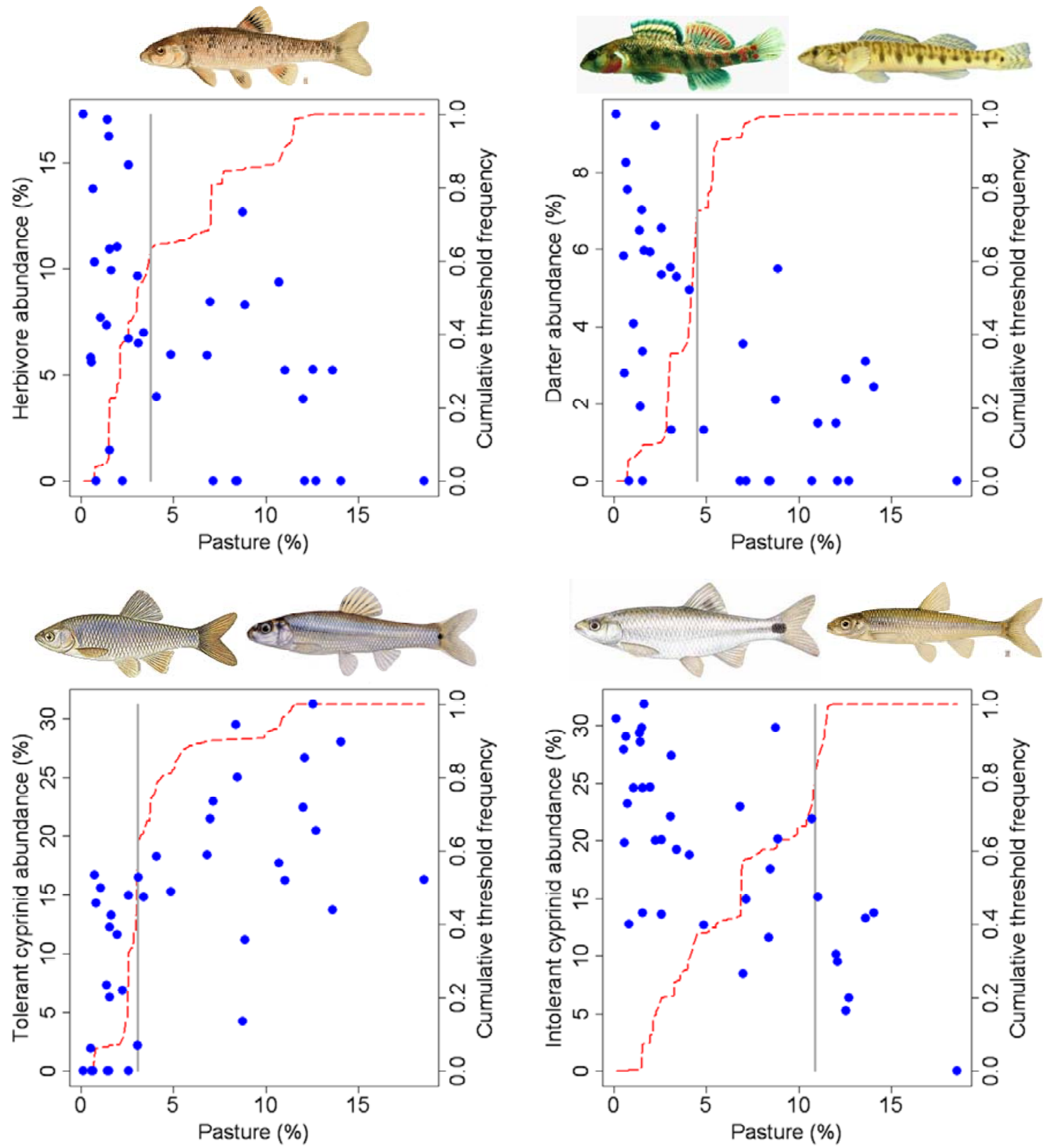

Figure 36. Results on nonparametric changepoint analysis using % pasture in the watershed as a predictor of threshold responses in the four proposed new fish metrics of nutrient-related reduction in biological integrity in Ecoregion 29.

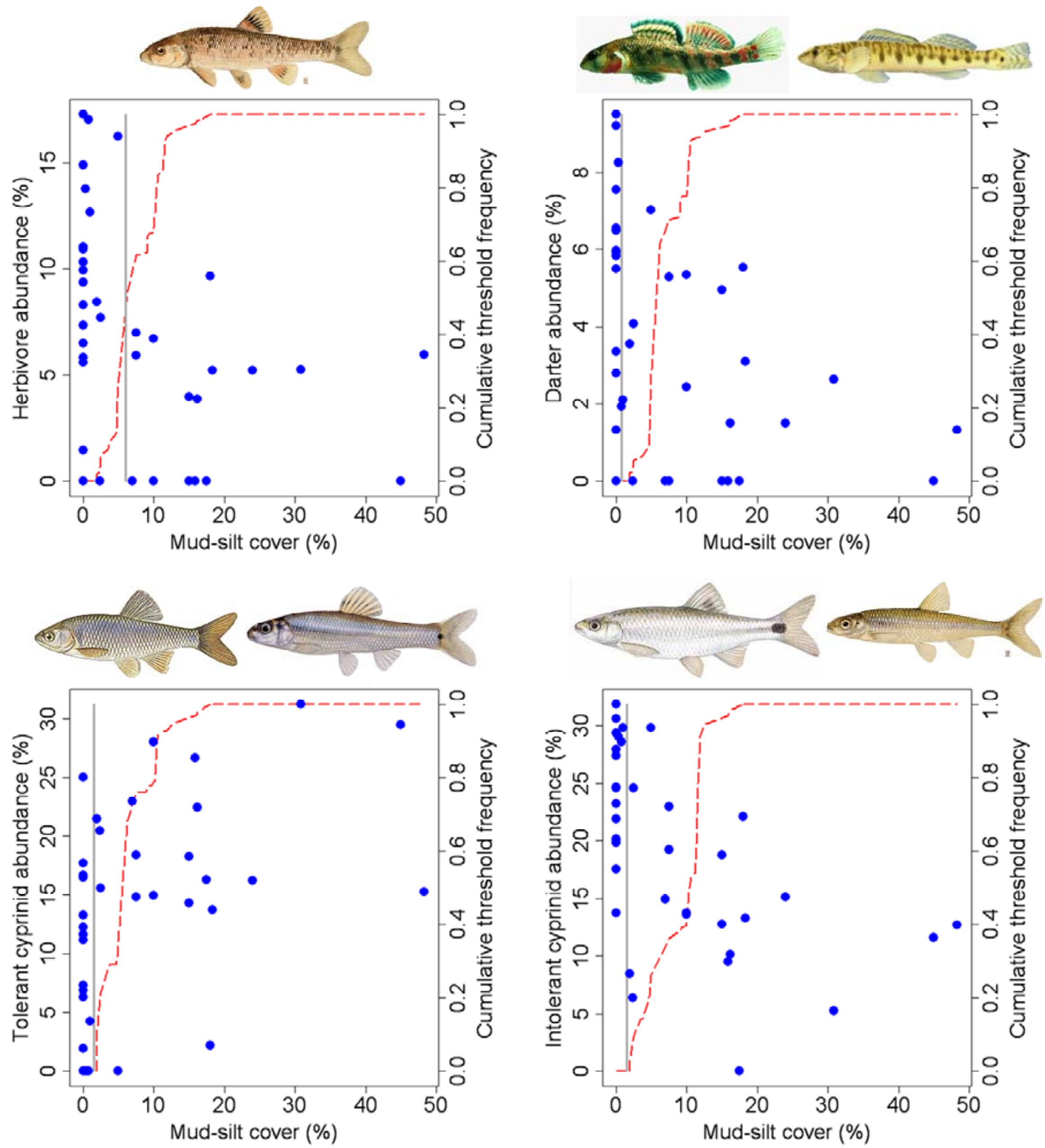

Figure 37. Results on nonparametric changepoint analysis using mud-silt cover (%) as a predictor of threshold responses in the four proposed new fish metrics of nutrient-related reduction in biological integrity in Ecoregion 29.

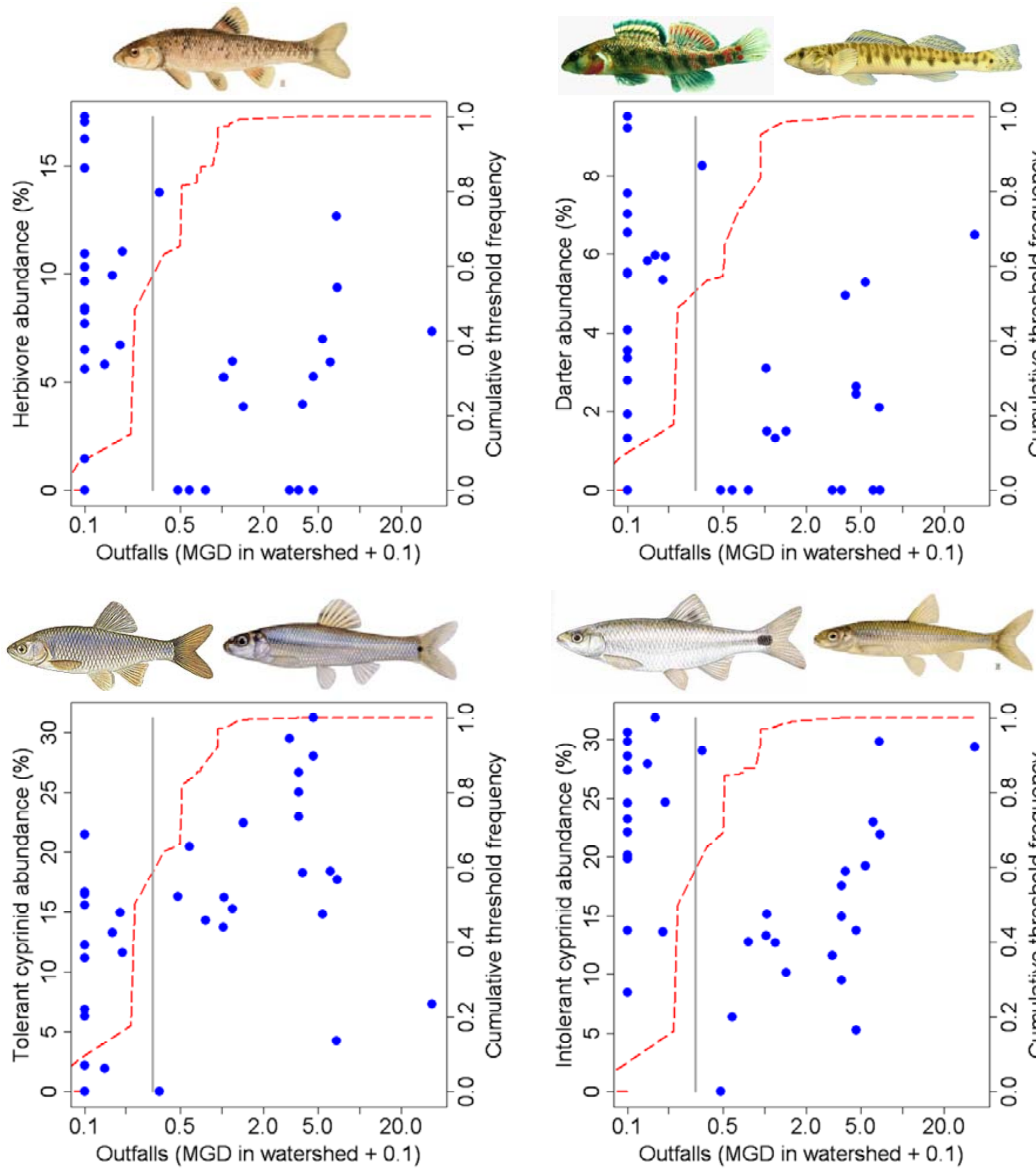

Figure 38. Results on nonparametric changepoint analysis using outfalls (permitted discharege in MGD; not necessarily the actual discharge) as a predictor of threshold responses in the four proposed new fish metrics of nutrient-related reduction in biological integrity in Ecoregion 29.

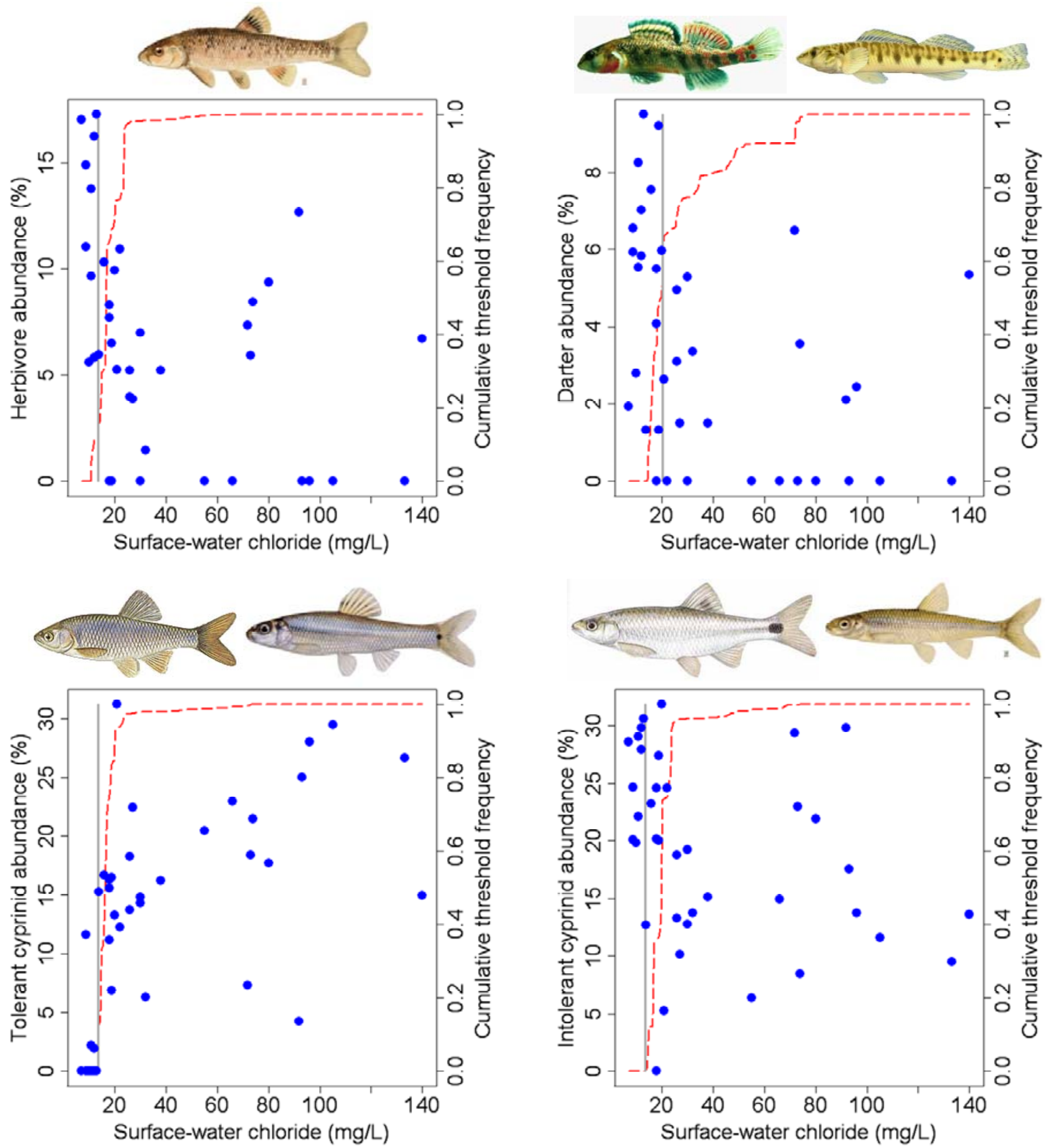

Figure 39. Results on nonparametric changepoint analysis using surface water chloride as a predictor of threshold responses in the four proposed new fish metrics of nutrient-related reduction in biological integrity in Ecoregion 29.

Table 4. Results of nonparametric changepoint analysis using nutrients and nutrient-related predictors of threshold responses in fish community indicators of biological integrity in Ecoregion 29. See figures xx through xx for graphical display of most of these results.

					Bootstrap Threshold Quantiles				
Predictor	Fish metric	Response > obs. threshold	Threshold (obs.)	P value	10%	50%	90%	Mean < obs.	Mean > obs.
Total phosphorus (ug/L)	Darters (%)	Decline	19.68	0.0015	15.12	19.68	30.18	5.93	1.98
Total phosphorus (ug/L)	Grazing herbivore (%)	Decline	24.22	0.0044	22.62	24.22	30.18	10.23	4.07
Total phosphorus (ug/L)	Nutrient-intolerant cyprinids (%)	Decline	24.22	0.0061	17.03	24.22	30.18	24.43	15.20
Total phosphorus (ug/L)	Community index (nMDS 1)	Increase	30.18	0.0041	17.03	25.20	55.15	-0.43	0.48
Total phosphorus (ug/L)	Nutrient-tolerant cyprinids (%)	Increase	52.08	0.0052	21.43	55.85	59.97	9.51	20.35
Periphyton C:P (bulk)	Darters (%)	Increase	178.01	0.0057	170.90	183.72	334.89	1.47	4.86
Periphyton C:P (bulk)	Grazing herbivore (%)	Increase	178.01	0.0159	142.56	178.01	334.10	3.60	8.89
Periphyton C:P (bulk)	Nutrient-intolerant cyprinids (%)	Increase	183.72	0.0180	95.50	183.72	368.01	14.89	22.87
Periphyton C:P (bulk)	Community index (nMDS 1)	Decline	178.01	0.0039	105.97	178.01	189.99	0.53	-0.39
Periphyton C:P (bulk)	Nutrient-tolerant cyprinids (%)	Decline	170.90	0.0096	95.50	173.06	313.72	19.53	9.57
Pasture (%)	Darters (%)	Decline	4.47	0.0023	2.11	4.47	5.45	5.09	1.39
Pasture (%)	Grazing herbivore (%)	Decline	3.75	0.0273	1.52	3.08	11.03	8.95	4.12
Pasture (%)	Nutrient-intolerant cyprinids (%)	Decline	10.87	0.0020	2.10	6.89	11.37	21.73	9.17

Pasture (%)	Community index (nMDS 1)	Increase	3.08	0.0024	2.42	3.08	6.93	-0.50	0.45
Pasture (%)	Nutrient-tolerant cyprinids (%)	Increase	3.08	0.0007	2.42	3.08	3.71	6.84	19.50
Embeddedness (%)	Darters (%)	Decline	12.50	0.0265	14.91	20.83	30.00	6.32	2.78
Embeddedness (%)	Grazing herbivore (%)	Decline	18.33	0.0074	12.50	18.33	30.83	11.17	4.83
Embeddedness (%)	Nutrient-intolerant cyprinids (%)	Decline	28.33	0.0137	17.50	30.00	47.92	23.68	15.37
Embeddedness (%)	Community index (nMDS 1)	Increase	20.83	0.0130	16.67	20.83	30.00	-0.52	0.30
Embeddedness (%)	Nutrient-tolerant cyprinids (%)	Increase	20.83	0.0190	15.83	20.83	32.50	7.67	16.91
Mud-silt (%)	Darters (%)	Decline	0.83	0.0484	5.00	6.25	10.63	4.72	2.40
Mud-silt (%)	Grazing herbivore (%)	Decline	6.00	0.0187	3.75	6.25	11.67	8.84	3.67
Mud-silt (%)	Nutrient-intolerant cyprinids (%)	Decline	1.50	0.0020	3.50	10.42	11.88	24.47	14.25
Mud-silt (%)	Community index (nMDS 1)	Increase	1.50	0.0025	2.00	6.25	10.63	-0.50	0.45
Mud-silt (%)	Nutrient-tolerant cyprinids (%)	Increase	1.50	0.0096	2.00	6.25	12.75	8.37	18.12
Outfalls (MGD)	Darters (%)	Decline	0.31	0.0030	0.22	0.38	0.93	5.22	1.65
Outfalls (MGD)	Grazing herbivore (%)	Decline	0.31	0.0071	0.22	0.38	0.93	9.55	3.77
Outfalls (MGD)	Nutrient-intolerant cyprinids (%)	Decline	0.31	0.0149	0.22	0.31	0.93	23.18	15.00
Outfalls (MGD)	Community index (nMDS 1)	Increase	0.31	0.0021	0.22	0.23	0.93	-0.49	0.49
Outfalls (MGD)	Nutrient-tolerant cyprinids (%)	Increase	0.31	0.0028	0.09	0.93	1.13	7.93	19.08
Chloride (mg/L)	Darters (%)	Decline	20.50	0.0091	15.50	20.00	49.00	5.19	2.01
Chloride (mg/L)	Grazing herbivore (%)	Decline	13.50	0.0029	12.00	17.00	24.00	12.36	4.89
Chloride (mg/L)	Nutrient-intolerant cyprinids (%)	Decline	13.50	0.0263	15.00	20.50	24.00	25.86	16.99
Chloride (mg/L)	Community index (nMDS 1)	Increase	13.50	0.0013	14.00	17.00	20.50	-0.91	0.28
Chloride (mg/L)	Nutrient-tolerant cyprinids (%)	Increase	13.50	0.0005	14.00	15.00	19.00	1.75	17.15

CONCLUSIONS AND RECOMMENDATIONS

The weight of evidence provided in this report, particularly when coupled with the 2-year EPA Region 6 study on nutrient criteria development (Appendix B), implies that nutrient enrichment is a very probable cause of numerous biological changes in streams in Ecoregion 29. Fine-sediment runoff from pastures and overgrazed riparian zones also appears to be stressor that covaries strongly with moderate levels of P enrichment, suggesting that sediment-bound P from pasture runoff is a potential source of enrichment. The highest levels of P enrichment are clearly associated with waste-water treatment plant outfalls. Streams with high volumes of effluent discharge host markedly different biota than relatively unenriched streams.

Specific findings of this study that have important implications for nutrient criteria development and biological assessment methods include the following:

- Because of the overwhelming evidence in this report and in King et al. (2009; Appendix B) of consistent biological changes in streams with > 20 ug/L TP, the current laboratory method used by TCEQ for determining total phosphorus (TP) should be modified to measure lower levels of TP than the current LOD of 50 ug/L. The BU method (Appendix C), which utilizes a Lachat Quik-chem 8500 flow-injection autoanalyzer with a 360 place autosampler, has a lab MDL of around 3.6 ug/L (recomputed based on each run) and has been used in numerous other labs across the country for detecting low levels of TP.

- The TCEQ method for computing total nitrogen based on the addition of nitrate-nitrite-N, ammonia-N and Kjeldahl N analytes yields results that are quite similar to the BU method of measuring total N in one analysis. However, at low levels of TN, the TCEQ method may overestimate TN if the LOD value for one or more of the component analytes is computed as part of the total. Most of the significant biological changepoints in response to TN were detected ranged from 261 to 440 ug/L, thus laboratory methods should ensure that levels at or below this range are within LODs.

- Periphyton nutrient content is a very robust and sensitive indicator of nutrient status in streams in Ecoregion 29, where limestone gravel, cobble, and bedrock are the dominant substrates. It was also very strongly related to nonlinear changes in algal species composition and fish community structure. We recommend that periphyton C, N and P content be considered as an integrative measure of stream nutrient status, and a strong predictor of biological changes in hard-bottomed streams.

- However, in soft-bottom streams of Ecoregions 32 and 33, periphyton nutrient content measured from sand/mud substrate was highly variable and did not correspond well to surface-water nutrients or changes in algal species composition. We do not recommend continued evaluation of sand/mud periphyton in these systems as an indicator of nutrient-related degradation, and suggest that alternative substrates such as wood or artificial substrates be considered in future studies.

- The existing TCEQ habitat assessment method that relies on 5 to 6 cross-sectional transects to assess stream habitat variables may not be sufficient for adequately characterizing cover of some important structural and functional elements of central Texas streams. Biofilm/microalgae thickness, submersed macrophyte cover, filamentous algae cover, substrate composition, and sediment film thickness on substrate are several metrics that were found to be responsive to TP enrichment by King et al. (2009; Appendix B). However, these variables were assessed using a whole-reach zig-zag transect with 100 points of measurement, which provided a more comprehensive characterization of these often patchy variables than the TCEQ cross-section approach. We compared similar metrics used in the HQI survey (June-August 2008) to those of King et al. (June 2009) and found relatively weak correspondence between the two protocols (Figure 40, next page). However, some of the variance could have been due to differences in the day of sampling (protocols were not compared on the same day at each site). Nevertheless, we recommend that the TCEQ physical habitat assessment and associated HQI consider incorporate more direct measures of these variables into their assessments and consider a more extensive coverage of the reach, either by adding more cross-section transects for certain variables (e.g., EMAP uses 21 for substrate characterization) or adopting the 100-point zig-zag approach used by King et al. 2009. Once investigators are adequately trained, the 100-point method is relatively rapid to employ (1 investigator can complete the 100-point counts in 1-2 hours).

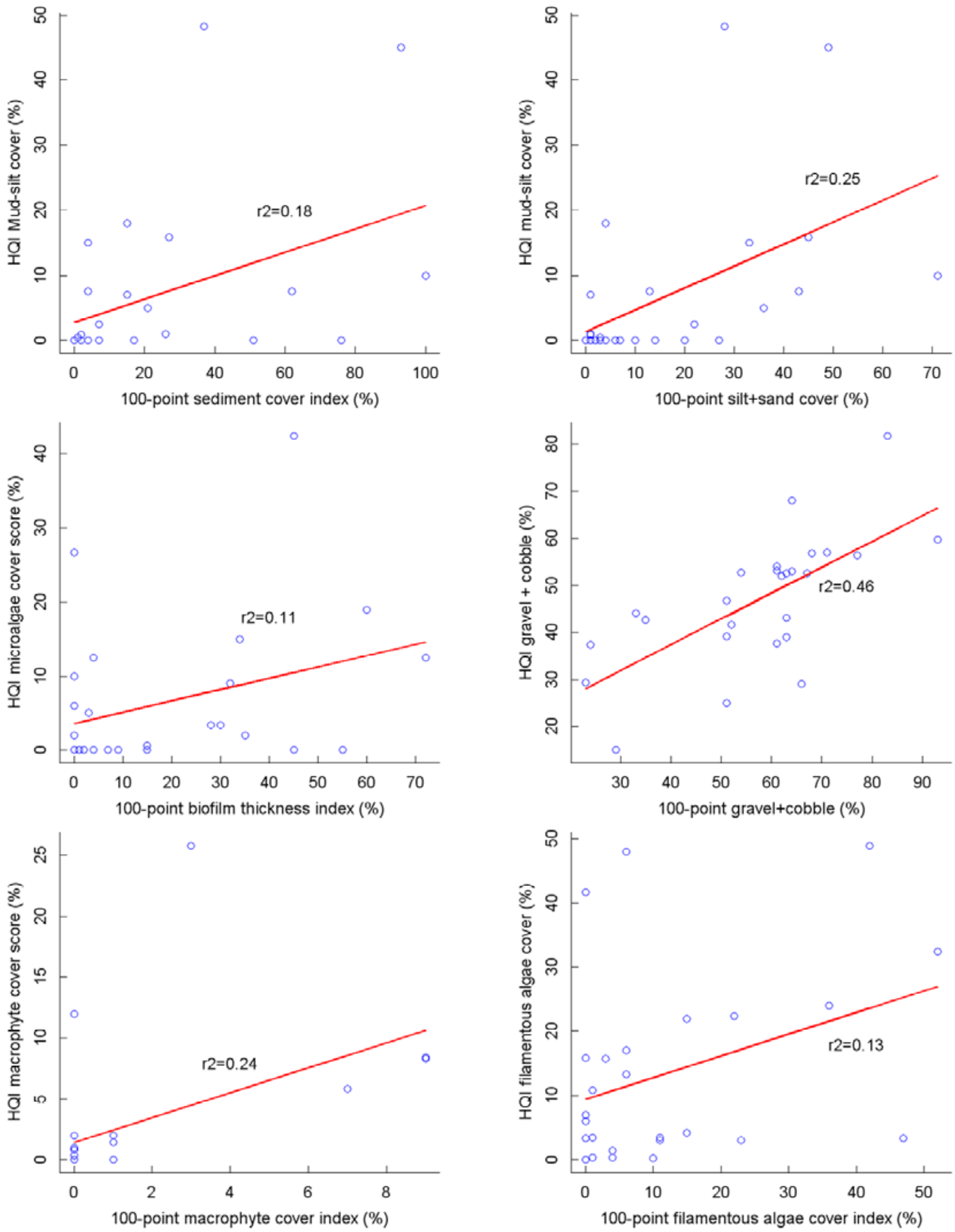

Figure 40. Comparison of fine sediment, gravel+cobble, filamentous algae, biofilm/microalgae, and macrophyte cover (% of reach) using the 100-point zig-zag transect method described in King et al. (2009) versus comparable metrics included in the TCEQ HQI method. Comparisons were made using the 26 stream locations sampled by King et al (2009) in June 2008 and the same locations sampled again in late June-August 2008 for the TCEQ HQI and Nutrient Indicators studies (Winemiller et al. 2009 and this report).

- As a practical compromise to adding some new physical habitat or algal/macrophyte metrics, we suggest that TCEQ consider reducing or elimating habitat measurements that are either never used in the existing HQI or were not shown to correspond to any biological changes in the 3 ecoregions, as recommended by Winemiller et al (2009).

- Periphyton chlorophyll-a and ash-free dry mass (AFDM) were not reliable indicators of nutrient enrichment in any ecoregion. This is not surprising given the shift from thick, calcareous periphyton comprised of cyanobacteria, diatoms, fungi, and bacteria to a community of pollution-tolerant diatoms and colonial/filamentous green algae consistently reported by King et al. (2009; Appendix B). Periphyton biomass is high in all of these streams, but the structure and function of the periphyton is very different in response to nutrient enrichment. The ratio of chlorophyll a to AFDM (CHLA:AFDM) did show a moderately strong response to TP enrichment in Ecoregion 29, and may be an indicator of significant functional changes in the periphyton as non-chlorophyll bearing organisms decline and are replaced by algae. This metric also consistently increased in response to TP in King et al. (2009; Appendix B).

- Surface-water variables related to particulates (chlorophyll-a, nonfiltrable and filtrable residue) also significantly increased in response to nutrients and may be useful indicators of nutrient-related degradation if found to exceed the reported thresholds in this report. However, some sites had low values for these variables even though sites had high nutrients and substantial changes in biological indicators, thus surface-water measures alone are not adequate for characterizing biological condition.

- Algal species composition was very strongly linked to surface water nutrients, particularly phosphorus, in Ecoregion 29, but was noisy and not related to any nutrient or nutrient related variables among the sand/mud algal samples from Ecoregions 32 and 33. We suggest that algal species composition may provide the most sensitive and direct measure of biological integrity in streams of Ecoregion 29. However, given the srong relationship to less costly and more easily measured predictors (surface-water nutrients, C:P content of periphyton, etc), these measures are likely to be strong surrogate variables for screening sites for potential biological degradation.

- Numerous algal species declined sharply in Ecoregion 29 in response to surface-water TP between 15 and 25 ug/L. Many other tolerant algae increased at TP between 20 and 50 ug/L. The significant threshold indicator species reported in this document, coupled with species lists provided in King et al. (2009; Appendix B) could be used in developing univariate metrics of nutrient enrichment for Ecoregion 29 streams.

- Fish communities were tightly coupled to the lower-trophic-level biological changes in streams of Ecoregion 29. Based on these and results described in Winemiller et al. (2009), we recommend five potential new fish metrics of nutrient or nutrient-related problems in streams of Ecoregion 29:
    - Fish community index (nMDS Axis 1). Site values are scores along the primary axis of variation in fish community structure from non-metric multidimensional scaling ordination of the 38 sites in Ecoregion 29 during summer 2008. Low (negative) scores represent sites that are most dissimilar from sites with high levels of outfalls, pasture, nutrients, chloride, and sediment (high, positive scores on axis 1).
    - Percent grazing herbivore abundance (*Campostoma anomalum*, central stoneroller). *Campostoma* was found to decline significantly in response to pasture, outfalls, embeddedness, and mud-silt in Winemiller et al. (2009), and additionally to chloride, TP, and C:P periphyton in this study. *Campostoma* plays a fundamental role in stream ecosystem processes in these streams by grazing on periphyton, recycling nutrients, exporting sediment, and as a primary food resource for native predator fishes such as spotted bass (*Micropterus punctalatus*).
    - Percent abundance of darters (*Etheostoma*). *Etheostoma spectabile* was the dominant benthic invertivore in clear-water, low nutrient streams in Ecoregion 29, but rapidly declined with increasing nutrient enrichment, sedimentation, chloride, and drivers of these stressors (outfalls, pasture). Other related species (*Percina* spp.) were too infrequently collected to determine statistical significance but

likely were negatively affected by these stressors as well and could be combined with this metric. Primarily riffle, crevice-dwelling fish, these fish are mechanistically linked to benthic processes in streams and are another key indicator of biological integrity in these ecosystems.

- Percent abundance of nutrient-intolerant cyprinids (*Cyprinella venusta, Notropis volucellus*). Blacktail shiners are common in most streams in Ecoregion 29, but their percent contribution to community structure clearly declined as nutrient enrichment and sedimentation increased. Mimic shiner was also sensitive to these stressors.
- Percent abundance of nutrient-tolerant cyprinids (*Cyprinella lutrensis, Pimephales vigilax*). Both of these species showed sharp increases in abundance with nutrient enrichment, as indicated by TITAN. Although these are native species and contribute positively to "number of native cyprinids", a metric used in the TCEQ IBI, these species are in fact very tolerant of pollution and benefit from human alterations to streams. Red shiners have been shown through historical analysis of Brazos River seine data (T. Bonner, unpublished data) to have markedly increased in abundance in the past 30-50 years while other native cyprinds have declined, a phenomenon coincident with dam construction and water quality declines in the mainstem Brazos. Red shiner was found to be particularly prolific at sites below outfalls in this study. *Pimephales vigilax*, or bullhead minnow, is a close relative to the toxicological test organism *Pimephales promelas*, or fathead minnow, used because of its ease in reproduction and resistance to physiological stress. Bullhead minnows were only occasionally collected in low-nutrient streams, but were dominant in enriched streams.

In summary, when coupling results of this study with findings of King et al. (2009; Appendix B), there is a very high probability that streams in Ecoregion 29 exposed to surface-water TP levels exceeding 20 ug/L, and possibly 15 ug/L, will experience a strong biological response needing further investigation to establish thresholds for nutrient management, including loss of characteristic structure (periphyton and macrophytes), loss of numerous species (algae , macroinvertebrates (King et al. 2009), and fish), additions of species that are associated with eutrophication or disturbance, minimum dissolved oxygen levels unsuitable for supporting native fauna during low flows (King et al. 2009), and increase likelihood of nuisance algal growth that could limit the recreational use of streams (King et al. 2009). Streams exceeding 200-500 ug/L may represent another threshold of biological response, with more consistent nuisance algal growth and additional losses of algal, macroinvertebrate and fish species and replacement with species associated with poor water quality.

Additional research on algae and fish community responses to nutrient enrichment and sedimentation is needed in Ecoregions 32 and 33. Insufficient numbers of sites coupled with the poor quality of the sand/mud samples renders these results too uncertain for definitive recommendations. Future studies need to target a minimum of 30 sites per ecoregion and use a reconnaissance approach before selecting sites to ensure that enough sites with both very low and high nutrient levels are represented in the data set to allow indicator development and threshold detection.

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Appendix A1. Key to water chemistry and periphyton variable short names used throughout this document.

Variable	Description
TKN	Total Kjehldal Nitrogen, ug/L
NH3-N	Ammonia-nitrogen, surface water, ug/L
NO2NO3-N	Nitrite + nitrate-nitrogen, surface water, ug/L
PO4-P	Orthophosphate, surface water, ug/L
TN_TCEQ	Total nitrogen, TCEQ lab, (NH3-N + TKN + NO2NO3N)
TN_BU	Total nitrogen, Baylor lab surface water, ug/L
TP_TCEQ	Total phosphorus, TCEQ lab, surface water, ug/L
TP_BU	Total phosphorus, Baylor lab, surface water, ug/L
ALKALIN	Alkalinity, total, surface water, mg/L
CHLORIDE	Chloride, surface water, mg/L
FLOURIDE	Flouride, surface water, mg/L
TNONRESI	Total nonfiltrable residue, mg/L
VNONRESI	Volatile nonfiltrable residue, mg/L
TFILRESI	Total filterable residue, mg/L
CHLA_UGL	Chlorophyll-a, surface water, ug/L
C_ALG	Total carbon, organic fraction of periphyton, %
C_BULK	Total carbon, bulk periphyton, %
N_ALG	Total nitrogen, organic fraction of periphyton, %
N_BULK	Total nitrogen, bulk periphyton, %
P_ALG	Total phosphorus, organic fraction of periphyton, %
P_BULK	Total phosphorus, bulk periphyton, %
CN_ALG	Carbon:nitrogen ratio, OM fraction of periphyton
CN_BULK	Carbon:nitrogen ratio, bulk periphyton
CP_ALG	Carbon:phosphorus ratio, OM fraction of periphyton
CP_BULK	Carbon:phosphorus ratio, bulk periphyton
CP_SED	Carbon:phosphorus ratio, sed fraction of periphyton
NP_ALG	Nitrogen:phosphorus ratio, OM fraction of periphyton
NP_BULK	Nitrogen:phosphorus ratio, bulk periphyton
CHLA_M2	Chloophyll a, periphyton, mg/m2 (rock surface area)
AFDM_M2	Ash-free dry mass, periphyton, g/m2
CHL_AFDM	Chlorophyll-a:AFDM ratio, periphyton, mg/g

Appendix A2. Local-scale environmental variables measured in the HQI component of the study.

Category	Abbreviation	Variable
Habitat type	HAB_TYPE	Habitat type score (riffle, run, pool, or glide) averaged across transects
	NO_RIFF	Number of riffles in study reach
Substrate	BEDROCK	Percent of substrate that is bedrock
	LG_BLDR	Percent of substrate that is large boulders (>45 cm)
	SM_BLDR	Percent of substrate that is small boulders (25-45 cm)
	COBBLE	Percent of substrate that is cobble (6-25 cm)
	GRAVEL	Percent of substrate that is gravel (2-60 mm)
	SAND	Percent of substrate that is sand (0.06-2 mm)
	MUDSILT	Percent of substrate that is mud or silt (<0.06 mm)
	GRV_LRG	Percent of substrate that is gravel or larger
	EMBEDDED	Substrate embeddedness (percent of boulders and cobble covered in fine sediment)
Algae/macrophytes	ALGAE_AB	Abundance of algae in study reach (scored as abundant, common, rare, or absent)
	MCRPH_AB	Abundance of aquatic macrophytes in study reach (scored as abundant, common, rare, or absent)
Instream cover	STRM_COV	Visually estimated percent cover
	FILA_ALG	Percent of instream cover provided by filamentous algae
	MICRALG	Percent of instream cover provided by microalgae and biofilms
	MACRPHYT	Percent of instream cover provided by aquatic macrophytes
	LWD	Percent of instream cover provided by large woody debris
	SWD	Percent of instream cover provided by small woody debris
	ROOTS	Percent of instream cover provided by submerged roots
	OVR_VEG	Percent of instream cover provided by overhanging terrestrial vegetation
	UNDERCUT	Percent of instream cover provided by undercut banks
	LEAFPACK	Percent of instream cover provided by leaf packs
	BOULDER	Percent of instream cover provided by boulders and other large substrates
	ARTIFICL	Percent of instream cover provided by artificial objects (e.g., tires, cement blocks)
	COV_TYPE	Number of the above cover types present

Appendix A2, continued. Local-scale environmental variables used in this study.

Category	**Abbreviation**	**Variable**
Stream morphology	STRMBEND	Number of stream bends in study reach
	WELLBEND	Number of well-defined stream bends in study reach
	MODBEND	Number of moderately-defined stream bends in study reach
	POORBEND	Number of poorly-defined stream bends in study reach
	WETWIDTH	Wetted width of stream (averaged across transects)
	AVG_DEP	Average stream depth
	THAL_DEP	Thalweg depth (averaged across transects)
	POOL_WID	Maximum pool width
	POOL_DEP	Maximum pool depth
	VELDEPTH	Velocity/depth regime score (optimal, suboptimal, marginal, or poor)
Flow	FLOWSTAT	Flow status score (high, moderate, low, or no flow)
	DISCHARG	Discharge (instantaneous stream flow in ft^3/s)
Roots/woody debris	CWD_WET	Count of wetted coarse woody debris in study reach
	CWD_BKF	Count of dry coarse woody debris within bank-full stream width
	ROOT_WET	Count of wetted root wads in study reach
	ROOT_BKF	Count of dry root wads within bank-full stream width
Riparian buffer	BUFFER	Width of riparian buffer (averaged across transects)
	RIP_TREE	Percent of riparian vegetation consisting of trees
	RIP_SHRB	Percent of riparian vegetation consisting of shrubs
	RIP_GRAS	Percent of riparian vegetation consisting of grasses/forbs
	RIP_CULT	Percent of riparian vegetation consisting of cultivated fields
	OTHER	Percent of riparian vegetation consisting of other types
	CANOPY	Percent of stream shaded by tree canopy (measured with densitometer)
Aesthetics	AESTHET	Aesthetics score (wilderness, natural area, common setting, or offensive)
Bank characteristics	BNK_SLOP	Bank slope (averaged across transects)
	EROSION	Percentage of bank with evident or potential erosion
	SOIL_EXP	Percentage of exposed soil on banks
Water parameters	DO	Dissolved oxygen (mg/L)
	PH	pH
	SPCOND	Specific conductivity (µs)
	TEMP	Water temperature (°C)

Appendix A3. Watershed physiographic variables used in this study

Variable	Description
LAT_DS	Latitude, decimal degrees
LONG_DS	Longitude, decimal degrees
EcoLev3	Level 3 ecoregion
PRECIP	Mean annual precipitation, calculated for watershed
ELEV_M	Mean elevation
WSLOPE	Mean watershed slope
WSHEDKM2	Watershed area
DAMS_CT	Number of dams in watershed
OUT_MGD	Cumulative permitted outfall discharge rate within watershed (million gallons per day)
OUT_CT	Number of outfalls
RESV_CT	Number of reservoirs within watershed
RESV_PCT	% of land covered by reservoirs within watershed
WATER	% of land covered by water within watershed
DEV_TOT	% developed land
FOR_TOT	% forested land, including forested wetlands
SHRUB	% shrubland
GRASS	% grassland
PASTURE	% pasture
ROWCROP	% rowcrop
WET_TOT	% wetland
AG_TOT	% agriculture (crop + pasture)
IMP_PCT	% impervious cover
CNPY_PCT	% canopy cover

Appendix A4. Species codes for fish collected among the 64 stream sites in 2008.

CODE	SPECIES	FAMILY	ORDER
AMEIMELA	*Ameiurus melas*	Ictaluridae	Siluriformes
AMEINATA	*Ameiurus natalis*	Ictaluridae	Siluriformes
APHRSAYA	*Aphredoderus sayanus*	Aphredoderidae	Percopsiformes
APLOGRUN	*Aplodinotus grunniens*	Sciaenidae	Perciformes
ASTYMEXI	*Astyanax mexicanus*	Characidae	Cypriniformes
ATRASPAT	*Atractosteus spatula*	Lepisosteidae	Semionotiformes
CAMPANOM	*Campostoma anomalum*	Cyprinidae	Cypriniformes
CARPCARP	*Carpiodes carpio*	Catostomidae	Cypriniformes
CYPRCARP	*Cyprinus carpio*	Cyprinidae	Cypriniformes
CYPRLUTR	*Cyprinella lutrensis*	Cyprinidae	Cypriniformes
CYPRVENU	*Cyprinella venusta*	Cyprinidae	Cypriniformes
DOROCEPI	*Dorosoma cepedianum*	Clupeidae	Clupeiformes
DOROPETE	*Dorosoma petenense*	Clupeidae	Clupeiformes
ERIMSUCE	*Erimyzon sucetta*	Catostomidae	Cypriniformes
ESOXAMER	*Esox americanus vermiculatus*	Esocidae	Esociformes
ETHECHLO	*Etheostoma chlorosomum*	Percidae	Perciformes
ETHEGRAC	*Etheostoma gracile*	Percidae	Perciformes
ETHESPEC	*Etheostoma spectabile*	Percidae	Perciformes
FUNDNOTA	*Fundulus notatus*	Fundulidae	Cyprinodontiformes
FUNDZEBR	*Fundulus zebrinus*	Fundulidae	Cyprinodontiformes
GAMBAFFI	*Gambusia affinis*	Poecilidae	Cyprinodontiformes
HYBONUCH	*Hybognathus nuchalis*	Cyprinidae	Cypriniformes
ICTAPUNC	*Ictalurus punctatus*	Ictaluridae	Siluriformes
ICTIBUBA	*Ictiobus bubalus*	Catostomidae	Cypriniformes
LABISICC	*Labidesthes sicculus*	Atherinidae	Atheriniformes
LEPIOCUL	*Lepisosteus oculatus*	Lepisosteidae	Semionotiformes
LEPIOSSE	*Lepisosteus osseus*	Lepisosteidae	Semionotiformes
LEPOAURI	*Lepomis auritus*	Centrarchidae	Perciformes
LEPOCYAN	*Lepomis cyanellus*	Centrarchidae	Perciformes
LEPOGULO	*Lepomis gulosus*	Centrarchidae	Perciformes
LEPOHUMI	*Lepomis humilus*	Centrarchidae	Perciformes
LEPOMACR	*Lepomis macrochirus*	Centrarchidae	Perciformes
LEPOMEGA	*Lepomis megalotis*	Centrarchidae	Perciformes
LEPOMICR	*Lepomis microlophus*	Centrarchidae	Perciformes
LEPOMINI	*Lepomis miniatus*	Centrarchidae	Perciformes
LEPOSPP	*Lepomis spp.*	Centrarchidae	Perciformes
LYTHFUME	*Lythrurus fumeus*	Cyprinidae	Cypriniformes
LYTHUMBR	*Lythrurus umbratilis*	Cyprinidae	Cypriniformes
MENIBERY	*Menidia beryllina*	Atherinidae	Atheriniformes
MICRPUNC	*Micropterus punctatus*	Centrarchidae	Perciformes
MICRSALM	*Micropterus salmoides*	Centrarchidae	Perciformes
MINYMELA	*Minytrema melanops*	Catostomidae	Cypriniformes
MOROCHRY	*Morone chrysops*	Moronidae	Perciformes
MOXOCONG	*Moxostoma congestum*	Catostomidae	Cypriniformes
MOXOPOEC	*Moxostoma poecilurum*	Catostomidae	Cypriniformes
MUGICEPH	*Mugil cephalus*	Mugilidae	Perciformes
NOTECRYS	*Notemigonus crysoleucas*	Cyprinidae	Cypriniformes

NOTRATRO	*Notropis atrocaudalis*	Cyprinidae	Cypriniformes
NOTRBUCH	*Notropis buchanani*	Cyprinidae	Cypriniformes
NOTRTEXA	*Notropis texanus*	Cyprinidae	Cypriniformes
NOTRVOLU	*Notropis volucellus*	Cyprinidae	Cypriniformes
NOTUGYRI	*Noturus gyrinus*	Ictaluridae	Siluriformes
NOTUNOCT	*Noturus nocturnus*	Ictaluridae	Siluriformes
OPSOEMIL	*Opsopoeodus emiliae*	Cyprinidae	Cypriniformes
PERCCARB	*Percina carbonaria*	Percidae	Perciformes
PERCMACR	*Percina macrolepida*	Percidae	Perciformes
PERCSCIE	*Percina sciera*	Percidae	Perciformes
PIMEVIGI	*Pimephales vigilax*	Cyprinidae	Cypriniformes
POMOANNU	*Pomoxis annularis*	Centrarchidae	Perciformes
POMONIGR	*Pomoxis nigromaculatus*	Centrarchidae	Perciformes
PYLOOLIV	*Pylodictis olivaris*	Ictaluridae	Siluriformes

Appendix A5. Species codes for algae collected among the 64 stream sites in 2008.

Type	TAXON_ID	SPECIES
Diatom	ACbiasol	*Achnanthes biassolettiana*
Diatom	ACcoarct	*Achnanthes coarctata*
Diatom	AClanapi	*Achnanthes lanceolata var. apiculata*
Diatom	ACploens	*Achnanthes ploenensis*
Diatom	ADbryoph	*Adlafia bryophila*
Diatom	AHexigum	*Achnanthidium exiguum*
Diatom	AHminuti	*Achnanthidium minutissimum*
Diatom	ALpelluc	*Amphipleura pellucida*
Diatom	AMbullat	*Amphora bullatoides*
Diatom	AMcoffea	*Amphora coffeaeformis*
Diatom	AMinarie	*Amphora inariensis*
Diatom	AMlibyca	*Amphora libyca*
Diatom	AMmontan	*Amphora montana*
Diatom	AMovalis	*Amphora ovalis*
Diatom	AMpedcls	*Amphora pediculus*
Diatom	AMsabina	*Amphora sabiniana*
Diatom	AMveneta	*Amphora veneta*
Diatom	ANcostat	*Anomoeoneis costata*
Diatom	ANsphaer	*Anomoeoneis sphaerophora*
Diatom	ANsphcos	*Anomoeoneis sphaerophora cf. costata*
Diatom	ATnorman	*Actinocyclus normanii*
Diatom	AUalpige	*Aulacoseira alpigena*
Diatom	AUambig	*Aulacoseira ambigua*
Diatom	AUgranlt	*Aulacoseira granulata*
Diatom	AUgrnang	*Aulacoseira granulata var. angustissima*
Diatom	AUsp	*Aulacoseira sp.*
Diatom	BApardxa	*Bacillaria paradoxa*
Diatom	BMcircum	*Biremis circumtexta*
Diatom	BMlucns	*Biremis lucens*
Diatom	BRvitrea	*Brachyseira vitrea*
Diatom	CAaeroph	*Caloneis aerophila*
Diatom	CAbacill	*Caloneis bacillum*
Diatom	CAschuma	*Caloneis schumanniana*
Diatom	CAsilicu	*Caloneis silicula*
Diatom	CCpedcls	*Cocconeis pediculus*
Diatom	CCplacen	*Cocconeis placentula*
Diatom	CCplapse	*Cocconeis placentula var. pseudolineata*
Diatom	CMaffins	*Cymbella affinis*
Diatom	CMamphic	*Cymbella amphicephala*
Diatom	CMcistul	*Cymbella neocistula*
Diatom	CMcymbif	*Cymbella cymbiformis*
Diatom	CMdelcat	*Cymbella delicatula*
Diatom	CMdelcat	*Encyonema delicatula*
Diatom	CMelgine	*Encyonema elginense*
Diatom	CMhusted	*Cymbella hustedtii*
Diatom	CMkolbei	*Cymbella kolbei*
Diatom	CMlaevis	*Cymbella laevis*
Diatom	CMnavfrm	*Cymbella naviculiformis*

Diatom	CMpaucst	*Encyonema (Cymbella) paucistriata*
Diatom	CMpusill	*Cymbella pusilla*
Diatom	CMtriang	*Cymbella triangulum*
Diatom	CMtumida	*Cymbella tumida*
Diatom	CPcrucic	*Capartogramma crucicula*
Diatom	CQsoehas	*Chamaepinnularia soehrensis var. hassiaca*
Diatom	CSdubius	*Cyclostephanos dubius*
Diatom	CSinvisi	*Cyclostephanos invisitatus*
Diatom	CStholif	*Cyclostephanos tholiformis*
Diatom	CTellipt	*Cymatopleura elliptica*
Diatom	CTsolea	*Cymatopleura solea*
Diatom	CYatomus	*Cyclotella atomus*
Diatom	CYdisuni	*Cyclotella distinguenda var. unipunctata*
Diatom	CYmenegh	*Cyclotella meneghiniana*
Diatom	CYmichig	*Cyclotella michiganiana*
Diatom	CYocella	*Cyclotella ocellata*
Diatom	CYstelli	*Cyclotella stelligera*
Diatom	DEkuetzi	*Denticula kuetzingii*
Diatom	DEsubtil	*Denticula subtilis*
Diatom	DIconfer	*Diadesmis confervacea*
Diatom	DIconten	*Diadesmis contenta*
Diatom	DPellipt	*Diploneis elliptica*
Diatom	DPmargin	*Diploneis marginestriata*
Diatom	DPoblong	*Diploneis oblongella*
Diatom	DPpsudov	*Diploneis pseudovalis*
Diatom	DPpuella	*Diploneis puella*
Diatom	ECminutu	*Encyonema minutum*
Diatom	ECneomul*	*Encyonema neomuelleri*
Diatom	ECprostr	*Encyonema prostratum*
Diatom	ECsilesi	*Encyonema silesiacum*
Diatom	ECtriang	*Encyonema triangulum*
Diatom	EPadnata	*Epithemia adnata*
Diatom	EPsorex	*Epithemia sorex*
Diatom	EPturgid	*Epithemia turgida*
Diatom	ESflexel	*Eucocconeis flexella*
Diatom	EUbilun	*Eunotia bilunaris*
Diatom	EUpectin	*Eunotia pectinalis*
Diatom	EYevergl	*Encyonopsis evergladianum*
Diatom	EYmicroc	*Encyonopsis microcephala*
Diatom	FAinsoc	*Fallacia insociabilis*
Diatom	FAsubham	*Fallacia subhamulata*
Diatom	FAtener2	*Fallatia tenera*
Diatom	FRcapuci	*Fragilaria capucina*
Diatom	FRellptc	*Fragilaria elliptica*
Diatom	FRfascic	*Fragilaria fasciculata*
Diatom	FRleptos	*Fragilaria leptostauron*
Diatom	FRnanan	*Fragilaria nanana*
Diatom	FRtenera	*Fragilaria tenera*
Diatom	FSrhoCAF	*Frustulia rhomboides*
Diatom	FSvulgar	*Frustulia vulgaris*

Diatom	GEaiken	*Geissleria aikenensis*
Diatom	GEdecu	*Geisleria decussis*
Diatom	GEthingv	*Geisleria thingvallae*
Diatom	GMgrovei	*Gomposphenia grovei*
Diatom	GMlinfor	*Gomphosphenia lingulatiformis*
Diatom	GNexigua	*Gomphonitzschia exigua*
Diatom	GOacumin	*Gomphonema acuminatum*
Diatom	GOaffine	*Gomphonema affine*
Diatom	GOangstt	*Gomphonema angustatum*
Diatom	GOangust	*Gomphonema angustum*
Diatom	GOclavat	*Gomphonema clavatum*
Diatom	GOgracil	*Gomphonema gracile*
Diatom	GOinsign	*Gomphonema insigne*
Diatom	GOintvib	*Gomphonema intricatum var vibrio*
Diatom	GOmaclau	*Gomphonema maclaughlinii*
Diatom	GOmexica	*Gomphonema mexicanum*
Diatom	GOparvul	*Gomphonema parvulum*
Diatom	GOpumilu	*Gomphonema pumilum*
Diatom	GOtrunca	*Gomphonema truncatum*
Diatom	GYacumin	*Gyrosigma acuminatum*
Diatom	GYeximum	*Gyrosigma eximium*
Diatom	Gynodfrm	*Gyrosigma nodiferium*
Diatom	GYobtusa	*Gyrosigma obtusatum*
Diatom	HAamphio	*Hantzschia amphioxys*
Diatom	HAcapita	*Hippodonta capitata*
Diatom	HAdist	*Hantzschia distinctepunctata*
Diatom	HIhunga	*Hippodonta hungarica*
Diatom	KCambig	*Craticula ambigua*
Diatom	KCbude	*Craticula buderi*
Diatom	KCcuspid	*Craticula cuspidata*
Diatom	LUgoepp2	*Luticola goeppertiana*
Diatom	LUmutica	*Luticola mutica*
Diatom	LUundula	*Luticola undulata*
Diatom	MDcircul	*Meridion circulare*
Diatom	MEvarian	*Melosira varians*
Diatom	MSellipt	*Mastogloia elliptica*
Diatom	MSsmithi	*Mastogloia smithii*
Diatom	MYatomus	*Mayamaea atomus*
Diatom	NAcaprad	*Navicula capitatoradiata*
Diatom	NAcarioc	*Navicula cariocincta*
Diatom	NAcfstr	*Navicula cf. striolata*
Diatom	NAcircum	*Navicula circumtexta*
Diatom	NAcrypto	*Navicula cryptocephala*
Diatom	NAcryten	*Navicula cryptotenella*
Diatom	NAerifga	*Navicula erifuga*
Diatom	NAgermii	*Navicula germainii*
Diatom	NAingua	*Navicula ingenua*
Diatom	NAkotsch	*Navicula kotschyi*
Diatom	NAlancel	*Navicula lanceolata*
Diatom	NAlatrpn	*Navicula(Geisleria) lateropunctata*

Diatom	NAlatrpn	*Navicula lateropunctata*
Diatom	NAlibone	*Navicula libonensis*
Diatom	NAlucdia	*Navicula sublucidula*
Diatom	NAmenscl	*Navicula menisculus*
Diatom	EOminima	*Eolima minima*
Diatom	NAoblong	*Navicula oblonga*
Diatom	NAorangi	*Navicula orangiana*
Diatom	NAphylpt	*Navicula phyllepta*
Diatom	NAradios	*Navicula radiosa*
Diatom	NArecens	*Navicula recens*
Diatom	NAreichd	*Navicula reichardtiana*
Diatom	NAreichd	*Navicula reichardtiana*
Diatom	NArhynch	*Navicula rhynchocephala*
Diatom	NArostel	*Navicula rostellata*
Diatom	NAsancru	*Navicula sanctaecrucis*
Diatom	NAschdei	*Navicula schadei*
Diatom	NAschroe	*Navicula schroeterii*
Diatom	NAstroem	*Sellaphora stroemii*
Diatom	NAsubmin	*Fallacia subminuscula*
Diatom	NEOsubmin	*Fallatia (Eolima) subminuscula*
Diatom	NAsubpla	*Navicula (Placoneis) subplacentula*
Diatom	NAsubrhy	*Navicula subrhynchocephala*
Diatom	NAsuec	*Navicula suecorum*
Diatom	NAsymtrc	*Navicula symmetrica*
Diatom	NAtenell	*Navicula tenelloides*
Diatom	NAtripun	*Navicula tripunctata*
Diatom	NAtrivis	*Navicula trivialis*
Diatom	NAveneta	*Navicula veneta*
Diatom	NEamplia	*Neidium ampliatum*
Diatom	NEbisulc	*Neidium bisulcatum*
Diatom	NEdubium	*Neidium dubium*
Diatom	NIaeroph	*Nitzschia aerophila*
Diatom	NIamphib	*Nitzschia amphibia*
Diatom	NIampoid	*Nitzschia amphibioides*
Diatom	NIangtu	*Nitzschia angustatula*
Diatom	NIangust	*Nitzschia angustata*
Diatom	NIbremen	*Nitzschia bremensis*
Diatom	NIbrevis	*Nitzschia brevissima*
Diatom	NIcapite	*Nitzschia capitellata*
Diatom	NIclausi	*Nitzschia clausii*
Diatom	NIcoarct	*Nitzschia coarctata*
Diatom	NIcombal	*Nitzschia compressa var. balatonis*
Diatom	NIcompre	*Nitzschia compressa*
Diatom	NIdebili	*Nitzschia debilis*
Diatom	NIdentic	*Nitzschia denticula*
Diatom	NIdissip	*Nitzschia dissipata*
Diatom	NIfilifr	*Nitzschia filiformis*
Diatom	NIfrustu	*Nitzschia frustulum*
Diatom	NIgeitlr	*Nitzschia geitleri*
Diatom	NIincons	*Nitzschia inconspicua*

Diatom	NIliebrt	*Nitzschia liebethruthii*
Diatom	NIlinear	*Nitzschia linearis*
Diatom	NIlorenz	*Nitzschia lorenziana*
Diatom	NImicroc	*Nitzschia microcephala*
Diatom	NInana	*Nitzschia nana*
Diatom	NIobtusa	*Nitzschia obtusa*
Diatom	NIpalea	*Nitzschia palea*
Diatom	NIrecta	*Nitzschia recta*
Diatom	NIrevers	*Nitzschia reversa*
Diatom	NIscalpe	*Nitzschia scalpelliformis*
Diatom	NIsigma	*Nitzschia sigma*
Diatom	NIsintab	*Nitzschia sinuata var. tabellaria*
Diatom	NIsolita	*Nitzschia solita*
Diatom	NItropic	*Nitzschia tropica*
Diatom	NIvaldec	*Nitzschia valdecostata*
Diatom	NIvitrea	*Nitzschia vitrea*
Diatom	ORdendro	*Orthoseira dentroteres*
Diatom	PBprotr	*Parlibellus protracta*
Diatom	PCclemto	*Placoneis clementioides*
Diatom	PCconst	*Placoneis constans*
Diatom	PCparel	*Placoneis paraelginensis*
Diatom	PCplacen	*Placoneis placentula*
Diatom	PCseudo	*Placoneis pseudanglica*
Diatom	PDbrevis	*Pseudostaurosira brevistriata*
Diatom	PGlepidp	*Plagiotropis lepidoptera*
Diatom	PIappend	*Pinnularia appendiculata*
Diatom	PIboreal	*Pinnularia borealis*
Diatom	PIgibba	*Pinnularia gibba*
Diatom	PIinterr	*Pinnularia interrupta*
Diatom	PIlundii	*Pinnularia lundii*
Diatom	PImicros	*Pinnularia microstauron*
Diatom	PIobscur	*Pinnularia obscura*
Diatom	PIsubcap	*Pinnularia subcapitata*
Diatom	PIviridi	*Pinnularia viridis*
Diatom	PLdelica	*Pleurosigma delicatulum*
Diatom	PRlaevis	*Pleurosira laevis*
Diatom	PTlanapi* AClanapi	*Planothidium (Achnanthes) lanceolatum var. apiculata*
Diatom	PTlanceo	*Planothidium lanceolata*
Diatom	REsinuta	*Reimeria sinuata*
Diatom	ROabbre	*Rhoicosphenia abbreviata*
Diatom	RPbrebsn	*Rhopalodia brebissonii*
Diatom	RPgibba	*Rhopalodia gibba*
Diatom	RPgibbrl	*Rhopalodia gibberula*
Diatom	RPmuscul	*Rhopalodia musculus*
Diatom	RPoprlta	*Rhopalodia operculata*
Diatom	SFlaevis	*Sellaphora laevissima*
Diatom	SFpupula	*Sellaphora pupula*
Diatom	SFseminu	*Sellaphora seminulum*
Diatom	SNstrigos	*Seminavis strigosa*
Diatom	SRconstr	*Staurosira construens*

Diatom	SRconven	*Staurosira construens var. venter*
Diatom	SSanceps	*Stauroneis anceps*
Diatom	SSobtusa	*Stauroneis obtusa*
Diatom	SSphoeni	*Stauroneis phoenicentron*
Diatom	SSpssbob	*Stauroneis pseudosubobtusoides*
Diatom	SSsmithi	*Stauroneis smithii*
Diatom	STmedius	*Stephanodiscus medius*
Diatom	SUangust	*Surirella angusta*
Diatom	SUbreb	*Surirella brebissonii*
Diatom	SUelegan	*Surirella elegans*
Diatom	SUminuta	*Surirella minuta*
Diatom	SUovalis	*Surirella ovalis*
Diatom	SUspiral	*Surirella spiralis*
Diatom	SUsplen	*Surirella splendida*
Diatom	SUtenera	*Surirella tenera*
Diatom	SYacus	*Synedra acus*
Diatom	SYgoular	*Synedra goulardi*
Diatom	SYulna	*Synedra ulna*
Diatom	TEmusica	*Terpsinoe musica*
Diatom	THbrampt	*Thalassiosira bramaputrae*
Diatom	THnorden	*Thalassiosira nordenskioldii Cleve*
Diatom	THsp	*Thalassiosira sp.*
Diatom	THvisurg	*Thalassiosira visurgis*
Diatom	TYacumin	*Tryblionella acuminata*
Diatom	TYaeroph	*Tryblionella aerophila*
Diatom	TYapicul	*Tryblionella apiculata*
Diatom	TYcaldid	*Tryblionella calida*
Diatom	TYcfmarg	*Tryblionella cf. marginulata*
Diatom	TYdebili	*Tryblionella debilis*
Diatom	TYhungar	*Nitzschia hungarica*
Diatom	TYhungar	*Tryblionella hungarica*
Diatom	TYlevide	*Tryblionella levidensis*
Diatom	TYlittor	*Tryblionella littoralis*
Soft	AFCsp	*Aphanothece sp.*
Soft	ANBsp	*Anabaena sp.*
Soft	ANKfalca	*Ankistrodesmus falcatus*
Soft	ANKsp	*Ankistrodesmus sp*
Soft	CALsp	*Calothrix sp.*
Soft	CHC0AUL	*Chlorococcum sp.*
Soft	CHLsp	*Chlamydomonas sp.*
Soft	CHOsp	*Chroococcus sp.*
Soft	CHRsp	*Characium sp.*
Soft	CLAglomer	*Cladophora glomerata*
Soft	CLAsp	*Cladophora sp.*
Soft	CLOsp2	*Closterium sp.*
Soft	COEsp	*Coelastrum sp.*
Soft	COHsp	*Coelosphaerium sp.*
Soft	COSsp	*Cosmarium sp.*
Soft	CRUsp	*Crucigenia sp*
Soft	DESsp	*Desmidium sp.*

Soft	EUGacus	*Euglena acus*
Soft	EUGsp	*Euglena sp.*
Soft	EUTsp	*Eutreptia sp*
Soft	GLCsp	*Gloeocystis sp.*
Soft	GLHsp	*Glothece sp.*
Soft	GLKturf	*Gloeoskene turfosa*
Soft	KIRobesa	*Kirchneriella obesa*
Soft	KIRsp	*Kirchneriella sp.*
Soft	MERconvl	*Merismopedia convoluta*
Soft	MERglauc	*Merismopedia glauca*
Soft	MICsp	*Microcystis sp*
Soft	MOUsp	*Mougeotia sp.*
Soft	OEDsp	*Oedogonium sp.*
Soft	OOCsp	*Oocystis sp.*
Soft	OSCsp	*Oscillatoria sp.*
Soft	PEDboryn	*Pediastrum boryanum*
Soft	PEDsp	*Pediastrum sp*
Soft	PHAsp	*Phacus sp.*
Soft	RIVsp	Unknown Rivulariaceae
Soft	SCEabund	*Scenedesmus abundans*
Soft	SCEbijug	*Scenedesmus bijuga*
Soft	SCEdimor	*Scenedesmus dimorphus*
Soft	SCEquadr	*Scenedesmus quadricauda*
Soft	SCEsp	*Scenedesmus sp.*
Soft	SCRsetig	*Schroderia setigera*
Soft	SCZsp	*Schizothrix sp.*
Soft	SPHsp	*Sphaerocystis sp.*
Soft	SPIsp	*Spirogyra sp.*
Soft	SPLsp	*Spirulina sp.*
Soft	STAsp	*Staurastrum sp.*
Soft	SYCsp	*Synechococcus sp.*
Soft	TETminum	*Tetraedron minimum*
Soft	TETregul	*Tetraedron regulare*
Soft	TETsp	*Tetraedron sp.*
Soft	TRAsp	*Trachelomonas sp.*
Soft	TRIsp	*Tribonema sp.*
Soft	UNcent	Centric diatoms
Soft	UNpennte	Pennate diatoms
Soft	XCLalga	*Cladophoraceae*
Soft	XDFalga	Unidentified dinoflagellates
Soft	XEUsp	Unknown Euglenophyte sp.
Soft	XXAsp	Unknown alga sp.

Appendix A6. Taxa-specific results from Threshold Indicator Taxa Analysis (TITAN) on **algal species composition** in response to nutrient and nutrient-related stressors among 38 sites in **Ecoregion 29** during summer 2008. Only species that showed significant threshold declines or increases in response to predictors are included in this table. The observed (*Obs*) threshold value of predictors for each taxon is shown in bold, whereas lower (*10%*), middle (*50%*), and upper (*90%*) quantiles of 1,000 bootstraps represent measures of uncertainty around the observed threshold. *Z* represents the standardized indicator score from TITAN (larger numbers = stronger threshold response), *IndVal* is the unstandardized indicator score (scaled from 0-100%, with 100=perfect indicator). *Purity* is the relative consistency of the response direction among the 1,000 bootstraps (*purity* > 0.95 is significant). *P-value* is the likelihood of getting an equal or larger IndVal if the score were computed with random shuffling of the observed data (*P*<0.05 is significant). See Appendix A5 for full species names corresponding to *Taxon IDs*.

								Bootstrap threshold quantiles		
Predictor	Taxon ID	Threshold (obs)	Response > obs.	z	IndVal	*P*	Purity	10%	50%	90%
TP (ug/L)	COSsp	**19.68**	Decline	3.24	60.83	0.004	1.000	16.18	22.82	1069.33
TP (ug/L)	KIRsp	**18.23**	Decline	3.25	25.00	0.036	0.968	10.89	17.03	24.22
TP (ug/L)	MERglauc	**21.43**	Decline	6.79	75.84	0.004	1.000	16.18	19.68	28.95
TP (ug/L)	OSCsp	**16.18**	Decline	5.31	72.66	0.004	1.000	14.27	17.03	34.18
TP (ug/L)	PEDboryn	**18.23**	Decline	3.94	38.60	0.008	0.970	14.27	17.03	24.22
TP (ug/L)	SCZsp	**125.08**	Decline	3.41	53.13	0.004	0.968	30.18	125.08	1069.33
TP (ug/L)	GOmaclau	**15.30**	Decline	5.54	42.22	0.004	0.996	14.27	16.61	21.43
TP (ug/L)	GOintvib	**17.03**	Decline	6.04	51.61	0.004	1.000	12.44	17.03	24.22
TP (ug/L)	NAstroem	**21.43**	Decline	5.64	55.04	0.004	1.000	12.44	19.68	28.95
TP (ug/L)	BRvitrea	**16.18**	Decline	5.13	52.22	0.004	0.998	10.89	16.18	30.18
TP (ug/L)	CMlaevis	**13.42**	Decline	4.04	43.37	0.020	0.988	10.89	14.27	30.18
TP (ug/L)	NIampoid	**15.30**	Decline	3.11	37.15	0.016	0.978	14.27	19.05	52.08
TP (ug/L)	ALpelluc	**34.18**	Decline	3.46	28.57	0.012	0.982	13.42	24.22	44.68
TP (ug/L)	HAamphio	**18.23**	Decline	3.66	25.00	0.028	0.960	10.89	17.03	24.22
TP (ug/L)	NAcrypto	**14.27**	Decline	3.09	36.67	0.016	0.962	12.44	17.03	40.73
TP (ug/L)	CMdelcat	**17.03**	Decline	8.00	83.62	0.004	1.000	14.62	18.23	24.22
TP (ug/L)	SYacus	**16.18**	Decline	6.34	65.13	0.004	0.998	12.44	16.18	19.68

TP (ug/L)	GOclavat	**24.22**	Decline	6.56	63.98	0.004	1.000	14.62	19.68	28.95
TP (ug/L)	GOgracil	**26.78**	Decline	3.92	51.69	0.004	0.996	18.23	28.95	77.03
TP (ug/L)	NAradios	**27.77**	Decline	3.64	33.33	0.024	0.990	14.62	26.78	40.73
TP (ug/L)	FRcapuci	**24.22**	Decline	4.01	53.37	0.004	0.978	15.30	24.22	44.68
TP (ug/L)	CMaffins	**34.18**	Decline	4.31	48.22	0.004	0.972	16.18	28.95	52.08
TP (ug/L)	GOangstt	**21.43**	Decline	4.94	53.50	0.004	0.998	15.30	27.77	77.03
TP (ug/L)	CMkolbei	**40.73**	Decline	4.91	54.55	0.012	0.996	24.22	40.73	69.78
TP (ug/L)	EYevergl	**21.43**	Decline	6.45	73.80	0.004	1.000	19.05	26.78	40.73
TP (ug/L)	EYmicroc	**21.43**	Decline	6.24	66.95	0.004	1.000	16.10	19.68	40.73
TP (ug/L)	ACbiasol	**21.43**	Decline	4.44	62.28	0.004	0.980	17.03	26.78	52.08
TP (ug/L)	DEkuetzi	**21.43**	Decline	5.82	67.48	0.004	1.000	14.27	19.05	34.18
TP (ug/L)	AHminuti	**52.08**	Decline	6.40	76.47	0.004	1.000	19.05	44.68	125.08
TP (ug/L)	ECsilesi	**10.89**	Decline	2.91	71.27	0.004	0.958	12.44	34.18	770.33
TP (ug/L)	SYulna	**69.78**	Decline	3.78	65.87	0.004	0.958	28.95	77.03	1069.33
TP (ug/L)	ANKsp	**44.68**	Increase	4.06	46.27	0.004	0.960	28.95	44.68	932.17
TP (ug/L)	CHRsp	**30.18**	Increase	4.98	44.44	0.004	1.000	26.78	34.18	598.33
TP (ug/L)	SCEquadr	**932.17**	Increase	8.93	60.00	0.004	0.954	368.33	1069.33	1235.00
TP (ug/L)	XXAsp	**10.89**	Increase	2.80	70.59	0.020	0.970	12.44	17.03	932.17
TP (ug/L)	NAsubmin	**1235.00**	Increase	5.45	63.31	0.012	0.958	40.73	1069.33	1235.00
TP (ug/L)	AMveneta	**125.08**	Increase	6.36	50.00	0.004	0.996	61.90	368.33	1069.33
TP (ug/L)	GMgrovei	**125.08**	Increase	3.12	26.09	0.024	0.968	28.95	125.08	1235.00
TP (ug/L)	ROabbre	**69.78**	Increase	4.98	33.33	0.004	0.986	44.68	77.03	598.33
TP (ug/L)	FRellptc	**52.08**	Increase	4.99	40.94	0.004	0.988	28.95	69.78	1069.33
TP (ug/L)	TEmusica	**26.78**	Increase	2.83	28.57	0.028	0.964	19.05	27.77	187.50
TP (ug/L)	NItropic	**44.68**	Increase	3.64	26.67	0.024	0.980	28.95	52.08	1235.00
TP (ug/L)	NIcombal	**44.68**	Increase	6.02	46.67	0.004	0.996	30.18	52.08	125.08
TP (ug/L)	NIangtu	**30.18**	Increase	3.39	27.78	0.016	0.986	26.78	52.08	1069.33
TP (ug/L)	AMsabina	**44.68**	Increase	3.97	46.37	0.004	0.982	21.43	52.08	1235.00
TP (ug/L)	GMlinfor	**26.78**	Increase	4.88	42.86	0.004	1.000	23.94	44.68	368.33
TP (ug/L)	TYapicul	**26.78**	Increase	2.86	35.81	0.016	0.968	19.05	28.95	69.78

TP (ug/L)	FAtener2	**52.08**	Increase	5.35	42.86	0.004	1.000	30.18	52.08	125.08
TP (ug/L)	NIsolita	**1069.33**	Increase	3.49	58.80	0.020	0.990	19.05	40.73	1235.00
TP (ug/L)	PCplacen	**1235.00**	Increase	5.51	89.33	0.008	0.998	30.06	125.08	1235.00
TP (ug/L)	PRlaevis	**125.08**	Increase	7.53	83.50	0.004	1.000	44.68	77.03	598.33
TP (ug/L)	AMpedcls	**69.78**	Increase	5.20	66.56	0.004	1.000	18.23	44.68	187.50
TP (ug/L)	AHexigum	**40.73**	Increase	4.99	60.69	0.004	1.000	18.23	28.95	598.33
TP (ug/L)	HIhunga	**44.68**	Increase	6.55	59.75	0.004	0.994	30.18	52.08	187.50
TP (ug/L)	NAsancru	**34.18**	Increase	5.90	50.29	0.004	1.000	26.78	40.73	77.03
TP (ug/L)	DIconfer	**21.43**	Increase	7.60	81.10	0.004	1.000	18.23	24.22	77.03
TP (ug/L)	NIincons	**30.18**	Increase	6.46	69.20	0.004	1.000	16.18	21.43	34.18
TP (ug/L)	NIfrustu	**40.73**	Increase	4.46	59.56	0.004	0.992	18.23	30.18	598.33
TP (ug/L)	GOpumilu	**16.18**	Increase	3.58	56.89	0.008	0.964	13.42	17.03	69.78
TP (ug/L)	NArecens	**24.22**	Increase	6.16	68.71	0.004	1.000	19.05	26.78	40.73
TP (ug/L)	REsinuta	**69.78**	Increase	3.22	59.41	0.020	0.990	14.62	34.18	131.32
TP (ug/L)	NAkotsch	**28.95**	Increase	2.86	48.89	0.012	0.988	17.03	34.18	1069.33
TP (ug/L)	CYmenegh	**19.05**	Increase	3.95	63.65	0.004	0.990	16.18	24.22	125.08
TP (ug/L)	CCplacen	**17.03**	Increase	6.34	76.98	0.004	0.996	13.42	17.03	21.43
TP (ug/L)	AMlibyca	**30.18**	Increase	3.14	57.13	0.008	0.972	17.03	27.77	77.03
TP (ug/L)	GOparvul	**14.27**	Increase	6.10	87.47	0.004	1.000	10.89	14.62	24.22
TP (ug/L)	NIamphib	**10.89**	Increase	3.01	65.58	0.024	0.984	10.89	12.44	77.03
TN (ug/L)	MERglauc	**271.00**	Decline	3.62	60.35	0.012	0.998	261.83	295.67	490.67
TN (ug/L)	OSCsp	**490.67**	Decline	4.60	61.48	0.004	0.994	280.17	440.83	633.33
TN (ug/L)	NAstroem	**271.00**	Decline	4.70	55.80	0.008	1.000	249.67	295.67	525.83
TN (ug/L)	BRvitrea	**266.00**	Decline	4.18	51.54	0.008	0.996	238.83	280.17	490.67
TN (ug/L)	ALpelluc	**249.67**	Decline	3.74	44.66	0.008	0.992	225.33	266.00	525.83
TN (ug/L)	CMdelcat	**328.17**	Decline	5.17	54.22	0.004	0.982	266.00	328.17	525.83
TN (ug/L)	SYacus	**362.00**	Decline	4.72	46.18	0.008	0.990	261.83	328.17	455.45
TN (ug/L)	RPgibba	**362.00**	Decline	2.91	43.12	0.016	0.986	266.00	402.50	918.17
TN (ug/L)	GOangstt	**546.17**	Decline	3.27	42.21	0.012	0.996	238.83	454.67	918.17
TN (ug/L)	EYevergl	**384.67**	Decline	5.56	68.35	0.004	0.994	294.12	402.50	918.17

TN (ug/L)	EYmicroc	**328.17**	Decline	5.84	67.33	0.004	0.996	261.83	328.17	440.83
TN (ug/L)	ACbiasol	**295.67**	Decline	5.65	68.51	0.004	1.000	249.67	280.17	494.18
TN (ug/L)	DEkuetzi	**261.83**	Decline	4.92	72.56	0.004	0.998	248.58	271.00	800.17
TN (ug/L)	AHminuti	**328.17**	Decline	5.75	71.94	0.004	1.000	295.67	420.17	1195.50
TN (ug/L)	ECsilesi	**249.67**	Decline	3.17	68.76	0.012	0.982	238.83	280.17	1891.67
TN (ug/L)										
TN (ug/L)	CHRsp	**420.17**	Increase	4.01	36.36	0.012	0.998	384.67	462.50	918.17
TN (ug/L)	AMveneta	**5723.33**	Increase	4.87	63.03	0.004	0.992	462.50	1891.67	5723.33
TN (ug/L)	NIcombal	**440.83**	Increase	3.48	33.33	0.020	0.994	402.50	462.50	1016.00
TN (ug/L)	GMlinfor	**384.67**	Increase	2.99	37.50	0.016	0.988	328.17	440.83	2393.33
TN (ug/L)	NIsolita	**454.67**	Increase	3.79	41.80	0.004	0.994	362.00	458.58	2393.33
TN (ug/L)	PCplacen	**5723.33**	Increase	2.75	55.35	0.024	0.986	384.67	1195.50	5723.33
TN (ug/L)	PRlaevis	**1195.50**	Increase	6.81	68.44	0.004	1.000	633.33	1891.67	3603.33
TN (ug/L)	AMpedcls	**462.50**	Increase	4.75	60.12	0.004	0.998	384.67	462.50	918.17
TN (ug/L)	NAsancru	**440.83**	Increase	3.70	39.66	0.004	0.980	295.67	420.17	633.33
TN (ug/L)	DIconfer	**1891.67**	Increase	5.29	77.39	0.004	1.000	271.00	867.83	2393.33
TN (ug/L)	NIincons	**440.83**	Increase	5.21	63.99	0.004	1.000	271.00	420.17	806.93
TN (ug/L)	NArecens	**328.17**	Increase	3.61	56.35	0.008	0.990	271.00	384.67	1016.00
TN (ug/L)	GOparvul	**261.83**	Increase	3.59	68.08	0.004	1.000	238.83	295.67	867.83
C:P (bulk)	CHRsp	**183.72**	Decline	5.21	44.44	0.004	1.000	133.26	182.03	312.80
C:P (bulk)	SCEquadr	**124.70**	Decline	5.59	33.33	0.008	0.950	95.50	118.40	147.69
C:P (bulk)	AMveneta	**159.00**	Decline	5.09	38.46	0.008	0.996	100.55	124.70	170.90
C:P (bulk)	GMgrovei	**178.01**	Decline	2.79	25.00	0.040	0.976	110.49	134.04	183.72
C:P (bulk)	ROabbre	**165.95**	Decline	3.98	28.57	0.020	0.976	95.50	147.69	182.03
C:P (bulk)	FRellptc	**159.00**	Decline	4.78	44.31	0.004	1.000	95.50	147.69	191.55
C:P (bulk)	NIcombal	**170.90**	Decline	4.20	35.51	0.008	1.000	100.55	170.90	245.12
C:P (bulk)	THsp	**191.55**	Decline	3.13	26.32	0.044	0.990	118.40	182.03	334.10
C:P (bulk)	NIangtu	**183.72**	Decline	3.22	27.78	0.020	0.990	95.50	134.04	245.12
C:P (bulk)	GMlinfor	**182.03**	Decline	6.08	52.94	0.004	1.000	124.70	170.90	245.12

C:P (bulk)	FAtener2	**165.95**	Decline	3.96	32.68	0.008	0.996	95.50	165.95	245.12
C:P (bulk)	PCplacen	**126.22**	Decline	3.84	41.51	0.020	0.974	100.55	126.22	183.72
C:P (bulk)	PRlaevis	**159.00**	Decline	8.13	73.53	0.004	1.000	118.40	134.04	170.90
C:P (bulk)	AMpedcls	**334.10**	Decline	5.43	65.98	0.004	1.000	178.01	312.80	341.09
C:P (bulk)	AHexigum	**134.04**	Decline	2.78	51.31	0.024	0.960	95.50	159.00	350.49
C:P (bulk)	HIhunga	**165.95**	Decline	5.49	54.83	0.004	1.000	100.55	134.04	183.72
C:P (bulk)	NAsancru	**159.00**	Decline	4.34	44.46	0.004	1.000	110.49	182.03	338.25
C:P (bulk)	DIconfer	**134.04**	Decline	6.80	80.28	0.004	1.000	121.16	159.00	183.72
C:P (bulk)	NIincons	**334.10**	Decline	6.22	73.56	0.004	1.000	178.01	334.10	350.49
C:P (bulk)	NIfrustu	**334.10**	Decline	3.25	51.91	0.004	0.986	100.55	170.90	363.95
C:P (bulk)	NArecens	**183.72**	Decline	5.09	60.14	0.008	0.996	110.49	178.01	334.27
C:P (bulk)	REsinuta	**338.25**	Decline	5.28	69.94	0.004	0.998	331.97	350.49	406.22
C:P (bulk)	CYmenegh	**462.85**	Decline	2.78	63.64	0.020	0.994	110.49	191.55	406.22
C:P (bulk)	CCplacen	**335.81**	Decline	6.15	73.01	0.004	1.000	245.12	340.04	438.57
C:P (bulk)	GOparvul	**312.80**	Decline	5.42	67.53	0.004	1.000	191.55	338.25	462.85
C:P (bulk)	NIamphib	**406.22**	Decline	2.79	60.55	0.004	0.950	118.40	390.09	562.94
C:P (bulk)	KIRsp	**363.95**	Increase	3.68	27.27	0.028	0.950	191.55	363.95	462.85
C:P (bulk)	MERglauc	**312.80**	Increase	5.04	62.86	0.004	1.000	182.03	335.81	390.09
C:P (bulk)	OSCsp	**245.12**	Increase	3.71	54.74	0.004	0.958	159.00	191.55	368.01
C:P (bulk)	SCZsp	**191.55**	Increase	3.79	52.76	0.004	1.000	126.22	183.72	390.09
C:P (bulk)	GOmaclau	**462.85**	Increase	5.09	55.01	0.008	0.996	334.10	390.09	562.94
C:P (bulk)	GOintvib	**462.85**	Increase	5.70	71.27	0.004	0.998	334.10	438.57	562.94
C:P (bulk)	NAstroem	**368.01**	Increase	4.54	47.86	0.008	1.000	165.95	312.80	390.09
C:P (bulk)	BRvitrea	**245.12**	Increase	5.50	50.00	0.004	1.000	182.03	323.45	406.22
C:P (bulk)	CMlaevis	**562.94**	Increase	4.36	61.04	0.020	0.982	182.03	498.33	562.94
C:P (bulk)	NIampoid	**183.72**	Increase	2.26	28.06	0.048	0.952	165.26	323.45	562.94
C:P (bulk)	ALpelluc	**350.49**	Increase	2.46	27.93	0.016	0.972	165.26	334.10	376.04
C:P (bulk)	HAamphio	**350.49**	Increase	3.27	25.00	0.040	0.956	334.10	363.95	498.33
C:P (bulk)	CMdelcat	**334.10**	Increase	4.95	54.08	0.004	0.996	245.12	368.01	462.85
C:P (bulk)	SYacus	**245.12**	Increase	6.59	50.00	0.004	1.000	183.72	335.81	406.22

C:P (bulk)	GOclavat	**562.94**	Increase	2.79	74.97	0.036	0.980	134.04	334.10	498.33
C:P (bulk)	GOgracil	**390.09**	Increase	3.86	56.62	0.004	0.996	147.69	312.80	438.57
C:P (bulk)	CMaffins	**165.95**	Increase	3.47	40.94	0.012	0.960	134.04	182.03	462.85
C:P (bulk)	CMkolbei	**165.95**	Increase	3.31	41.87	0.012	0.986	126.22	170.90	334.10
C:P (bulk)	EYevergl	**182.03**	Increase	6.58	72.79	0.004	1.000	147.69	178.01	312.80
C:P (bulk)	EYmicroc	**312.80**	Increase	6.00	67.01	0.004	1.000	182.03	334.10	376.04
C:P (bulk)	PIgibba	**170.90**	Increase	4.00	39.13	0.008	0.996	165.26	191.55	438.57
C:P (bulk)	NAcryten	**340.04**	Increase	3.44	42.54	0.008	0.992	159.00	350.49	562.94
C:P (bulk)	DEkuetzi	**335.81**	Increase	7.49	75.32	0.004	1.000	182.03	335.81	363.95
C:P (bulk)	GOaffine	**390.09**	Increase	2.32	60.46	0.032	0.954	95.50	334.10	406.22
C:P (bulk)	AHminuti	**165.95**	Increase	6.18	74.83	0.004	0.996	124.70	165.95	183.72
C:P (OM)	CHRsp	**165.20**	Decline	7.50	54.90	0.004	0.996	153.44	165.20	177.05
C:P (OM)	GLKturf	**153.44**	Decline	3.53	33.49	0.036	0.984	137.69	155.31	180.64
C:P (OM)	SCEquadr	**147.31**	Decline	3.68	25.76	0.024	0.968	131.40	156.76	177.05
C:P (OM)	AMveneta	**147.31**	Decline	4.22	38.61	0.012	0.968	131.40	153.44	185.75
C:P (OM)	FRellptc	**147.31**	Decline	4.49	49.84	0.008	0.996	137.69	155.31	183.77
C:P (OM)	NIcombal	**137.69**	Decline	3.41	51.99	0.016	0.998	131.40	155.31	193.47
C:P (OM)	NAtrivis	**156.76**	Decline	4.21	35.64	0.008	0.978	147.31	161.63	182.03
C:P (OM)	NIangtu	**166.54**	Decline	4.89	35.71	0.004	0.992	147.31	165.20	177.05
C:P (OM)	GMlinfor	**216.60**	Decline	3.25	37.50	0.024	0.986	141.18	182.03	227.15
C:P (OM)	FAtener2	**156.76**	Decline	4.57	46.88	0.012	0.998	131.40	156.76	180.64
C:P (OM)	NIsolita	**141.57**	Decline	3.60	53.05	0.012	0.996	131.40	156.76	225.08
C:P (OM)	PRlaevis	**137.69**	Decline	4.32	66.41	0.012	0.996	137.69	165.37	193.47
C:P (OM)	AMpedcls	**225.08**	Decline	4.01	57.32	0.008	0.988	176.64	205.26	228.73
C:P (OM)	AHexigum	**156.76**	Decline	4.86	67.63	0.004	0.998	153.44	166.54	227.15
C:P (OM)	HIhunga	**169.17**	Decline	4.62	50.60	0.008	0.970	131.40	166.54	183.77
C:P (OM)	NAsancru	**182.03**	Decline	3.22	37.69	0.008	0.970	172.88	193.47	227.99
C:P (OM)	DIconfer	**225.08**	Decline	5.33	64.66	0.004	0.996	155.31	193.47	228.73
C:P (OM)	NIincons	**225.08**	Decline	6.11	73.68	0.004	1.000	177.05	216.60	244.77
C:P (OM)	NIfrustu	**225.08**	Decline	3.71	59.62	0.008	0.966	180.64	216.60	244.77

C:P (OM)	NArecens	**161.63**	Decline	4.61	61.85	0.004	1.000	156.76	182.03	227.99
C:P (OM)	CYmenegh	**216.60**	Decline	3.56	54.86	0.004	0.962	147.31	193.47	244.77
C:P (OM)	CCplacen	**225.08**	Decline	5.24	71.99	0.004	0.986	182.03	205.26	246.50
C:P (OM)	GOparvul	**227.15**	Decline	6.03	77.60	0.004	1.000	193.47	227.99	288.83
C:P (OM)	NIamphib	**288.83**	Decline	3.45	64.03	0.008	0.980	155.12	273.82	298.29
C:P (OM)	MERglauc	**225.08**	Increase	6.17	71.16	0.004	1.000	166.54	193.47	227.99
C:P (OM)	OSCsp	**244.77**	Increase	4.99	69.93	0.004	0.988	193.47	227.99	264.51
C:P (OM)	PEDboryn	**183.77**	Increase	4.20	33.33	0.012	0.998	182.03	216.60	264.51
C:P (OM)	SCZsp	**185.75**	Increase	2.92	52.32	0.004	0.970	155.12	185.75	227.15
C:P (OM)	GOmaclau	**216.60**	Increase	5.11	35.71	0.004	0.996	193.47	244.77	298.29
C:P (OM)	NAstroem	**227.99**	Increase	3.19	43.86	0.012	0.984	165.20	227.15	288.83
C:P (OM)	BRvitrea	**216.60**	Increase	4.27	41.35	0.008	0.998	166.54	216.60	288.83
C:P (OM)	CMdelcat	**225.08**	Increase	7.02	69.18	0.004	0.994	185.75	225.08	262.07
C:P (OM)	SYacus	**193.47**	Increase	6.66	56.25	0.004	1.000	182.03	216.60	288.83
C:P (OM)	GOclavat	**244.77**	Increase	4.36	59.30	0.004	0.964	172.88	227.15	273.82
C:P (OM)	GOgracil	**180.64**	Increase	6.28	64.96	0.004	1.000	166.54	182.03	216.60
C:P (OM)	NAradios	**182.03**	Increase	4.29	31.58	0.004	0.992	172.88	183.77	244.77
C:P (OM)	FRcapuci	**165.20**	Increase	4.09	56.33	0.004	0.974	155.31	166.54	227.99
C:P (OM)	EYevergl	**216.60**	Increase	4.32	61.76	0.004	0.988	155.31	193.47	262.07
C:P (OM)	EYmicroc	**216.60**	Increase	4.67	55.91	0.004	0.994	168.91	227.15	273.82
C:P (OM)	PIgibba	**225.08**	Increase	2.68	34.61	0.024	0.976	169.17	205.26	273.82
C:P (OM)	ACbiasol	**165.20**	Increase	3.37	54.25	0.016	0.990	161.63	227.15	295.89
C:P (OM)	NAcryten	**166.54**	Increase	3.41	41.95	0.012	0.978	156.76	169.17	288.83
C:P (OM)	DEkuetzi	**227.15**	Increase	4.01	59.99	0.008	0.994	152.83	205.26	244.77
C:P (OM)	AHminuti	**244.77**	Increase	4.67	68.79	0.004	1.000	141.57	205.26	262.07
C:P (OM)	ECsilesi	**273.82**	Increase	3.36	68.76	0.008	0.990	165.55	262.07	288.83
Pasture (%)	GLHsp	**3.08**	Decline	3.11	58.21	0.004	0.996	2.11	4.47	13.17
Pasture (%)	MERglauc	**2.58**	Decline	3.29	53.58	0.012	0.990	0.77	2.11	7.76

Pasture (%)	SCEbijug	**2.81**	Decline	4.17	43.30	0.004	0.982	1.58	2.81	4.47
Pasture (%)	SPHsp	**8.61**	Decline	3.59	53.94	0.004	0.956	2.81	8.61	11.53
Pasture (%)	GOmaclau	**2.11**	Decline	4.77	35.71	0.004	0.992	0.68	1.54	2.81
Pasture (%)	NAstroem	**5.83**	Decline	3.27	38.49	0.004	0.982	0.68	2.11	7.05
Pasture (%)	BRvitrea	**5.83**	Decline	4.25	40.91	0.004	1.000	0.68	1.58	7.05
Pasture (%)	NIampoid	**1.79**	Decline	5.75	42.01	0.004	1.000	0.77	1.54	3.08
Pasture (%)	CMdelcat	**0.77**	Decline	5.58	84.70	0.004	0.976	0.68	0.93	2.58
Pasture (%)	SYacus	**1.79**	Decline	5.29	49.49	0.004	1.000	0.68	1.52	3.75
Pasture (%)	GOclavat	**2.11**	Decline	5.29	57.64	0.004	0.998	0.77	1.54	3.08
Pasture (%)	GOgracil	**4.47**	Decline	4.44	53.63	0.004	0.996	1.54	3.26	8.42
Pasture (%)	CMkolbei	**3.08**	Decline	4.93	49.71	0.004	0.974	1.58	3.08	6.89
Pasture (%)	EYevergl	**0.77**	Decline	3.66	75.83	0.004	0.986	0.77	1.54	5.83
Pasture (%)	EYmicroc	**1.46**	Decline	6.14	72.39	0.004	0.994	0.77	1.46	2.58
Pasture (%)	NAcryten	**1.58**	Decline	3.32	45.34	0.016	0.990	1.39	2.11	7.05
Pasture (%)	DEkuetzi	**3.75**	Decline	3.54	54.69	0.012	0.990	0.77	2.81	8.42
Pasture (%)	AHminuti	**1.79**	Decline	4.89	65.57	0.004	0.986	1.46	2.42	5.83
Pasture (%)										
Pasture (%)	ROabbre	**7.76**	Increase	4.40	30.77	0.008	0.992	3.75	7.76	9.79
Pasture (%)	TEmusica	**12.64**	Increase	6.39	67.16	0.008	0.996	8.42	11.53	12.64
Pasture (%)	NIcombal	**7.76**	Increase	6.39	53.85	0.004	1.000	4.47	8.42	10.87
Pasture (%)	NIangtu	**11.53**	Increase	5.69	54.42	0.008	0.996	7.05	10.87	12.64
Pasture (%)	GMlinfor	**8.42**	Increase	6.86	60.67	0.004	0.996	6.89	8.61	12.33
Pasture (%)	TYapicul	**6.89**	Increase	5.48	48.99	0.004	0.998	3.75	8.42	12.36
Pasture (%)	NIangust	**9.79**	Increase	4.61	55.07	0.008	0.998	3.75	8.82	12.64
Pasture (%)	FAtener2	**6.89**	Increase	5.41	40.00	0.004	0.998	3.75	7.05	10.87
Pasture (%)	NIsolita	**12.64**	Increase	3.11	61.67	0.020	0.994	1.58	8.61	12.64
Pasture (%)	NAminima	**9.79**	Increase	4.54	53.49	0.008	0.994	1.58	8.42	12.07
Pasture (%)	PRlaevis	**5.83**	Increase	4.10	50.23	0.008	0.992	2.58	6.89	8.82
Pasture (%)	AMpedcls	**2.58**	Increase	4.21	51.80	0.004	1.000	1.79	3.75	11.53
Pasture (%)	TYlevide	**7.05**	Increase	3.96	38.56	0.012	0.988	2.58	7.05	10.87
Pasture (%)	AHexigum	**2.58**	Increase	3.20	51.42	0.016	0.992	0.93	2.42	9.79

Pasture (%)	HIhunga	**1.54**	Increase	3.67	44.44	0.008	1.000	1.58	6.89	12.07
Pasture (%)	NAsancru	**4.47**	Increase	5.54	50.29	0.004	1.000	2.79	5.83	9.79
Pasture (%)	DIconfer	**12.07**	Increase	4.37	74.93	0.004	1.000	1.46	4.47	12.07
Pasture (%)	NIincons	**10.87**	Increase	4.88	71.94	0.004	1.000	2.58	8.42	12.07
Pasture (%)	GOpumilu	**1.58**	Increase	3.11	52.52	0.012	0.988	1.39	2.58	9.79
Pasture (%)	DPellipt	**12.64**	Increase	3.21	68.42	0.020	0.960	1.54	6.89	12.64
Pasture (%)	NArecens	**10.87**	Increase	6.53	80.05	0.004	1.000	5.83	8.82	12.07
Pasture (%)	REsinuta	**3.75**	Increase	3.76	58.41	0.004	0.974	2.42	5.83	8.82
Pasture (%)	CCplacen	**4.47**	Increase	5.21	67.39	0.004	1.000	0.93	3.26	6.89
Pasture (%)	GOparvul	**1.21**	Increase	3.65	71.18	0.004	0.982	0.68	1.21	6.89
Pasture (%)	NIamphib	**0.93**	Increase	6.44	70.59	0.004	0.988	0.68	0.93	1.52
Mud-silt (%)	MERglauc	**0.00**	Decline	3.87	55.10	0.008	0.966	0.00	1.50	10.00
Mud-silt (%)	PEDboryn	**0.00**	Decline	3.74	37.91	0.008	0.986	0.00	2.20	7.50
Mud-silt (%)	SCEbijug	**0.00**	Decline	4.07	42.49	0.004	0.996	0.00	0.00	7.28
Mud-silt (%)	CMdelcat	**0.20**	Decline	4.42	48.20	0.004	0.994	0.00	0.92	7.50
Mud-silt (%)	SYacus	**0.62**	Decline	4.16	38.60	0.008	0.992	0.00	0.62	7.25
Mud-silt (%)	GOclavat	**7.25**	Decline	5.17	55.64	0.004	0.994	0.00	2.45	8.88
Mud-silt (%)	GOgracil	**8.75**	Decline	2.75	44.76	0.032	0.958	0.00	6.00	15.00
Mud-silt (%)	EYevergl	**6.00**	Decline	3.62	57.37	0.008	0.966	0.00	3.75	15.00
Mud-silt (%)	DEkuetzi	**7.25**	Decline	3.69	53.15	0.008	0.988	0.00	2.45	12.50
Mud-silt (%)	SYulna	**8.75**	Decline	3.71	63.68	0.004	0.968	0.00	7.50	15.42
Mud-silt (%)	GLKturf	**17.75**	Increase	6.32	47.83	0.004	0.952	3.75	17.75	27.42
Mud-silt (%)	CSdubius	**16.04**	Increase	5.61	37.50	0.008	0.950	10.00	16.04	18.17
Mud-silt (%)	NIangtu	**3.75**	Increase	3.65	29.41	0.004	0.996	2.20	7.50	15.42
Mud-silt (%)	TYapicul	**15.42**	Increase	2.97	44.99	0.028	0.950	2.20	16.04	21.17
Mud-silt (%)	CAsilicu	**18.17**	Increase	3.09	48.70	0.020	0.990	1.50	16.88	27.42
Mud-silt (%)	NAminima	**15.42**	Increase	4.32	56.09	0.004	0.962	3.75	15.00	17.75
Mud-silt (%)	AMpedcls	**8.75**	Increase	5.35	61.76	0.004	1.000	2.20	8.75	18.17
Mud-silt (%)	NAsancru	**21.17**	Increase	2.82	59.91	0.020	0.978	1.50	15.00	27.42

Mud-silt (%)	NIincons	**8.75**	Increase	5.18	68.54	0.008	1.000	0.92	7.25	15.00
Mud-silt (%)	GOpumilu	**3.75**	Increase	5.20	59.50	0.004	0.966	0.62	3.75	10.00
Mud-silt (%)	NArecens	**10.00**	Increase	6.15	71.83	0.008	0.996	3.75	10.00	16.04
Mud-silt (%)	REsinuta	**12.50**	Increase	5.09	70.16	0.004	0.996	6.00	12.50	16.04
Mud-silt (%)	CAbacill	**17.75**	Increase	4.27	72.70	0.008	0.984	7.13	16.88	21.17
Mud-silt (%)	CCplacen	**0.00**	Increase	4.46	63.69	0.008	1.000	0.00	7.25	15.00
Mud-silt (%)	GOparvul	**8.75**	Increase	3.75	61.63	0.012	0.990	0.00	7.25	15.00
Outfalls (MGD)	SCZsp	**5.69**	Decline	3.95	55.45	0.004	0.994	0.01	4.55	6.41
Outfalls (MGD)	UNpennte	**4.92**	Decline	3.89	59.63	0.004	0.968	1.22	4.15	5.69
Outfalls (MGD)	GOmaclau	**0.32**	Decline	3.12	26.32	0.036	0.968	0.01	0.18	0.94
Outfalls (MGD)	CTellipt	**0.01**	Decline	3.86	31.83	0.016	0.966	0.01	0.06	0.44
Outfalls (MGD)	FRtenera	**0.08**	Decline	3.84	31.25	0.008	0.996	0.01	0.03	0.32
Outfalls (MGD)	GOintvib	**0.94**	Decline	2.85	30.43	0.016	0.982	0.01	0.44	1.22
Outfalls (MGD)	EPturgid	**0.94**	Decline	2.71	30.43	0.044	0.992	0.01	0.44	1.22
Outfalls (MGD)	NAstroem	**0.08**	Decline	5.41	51.11	0.004	1.000	0.01	0.08	0.81
Outfalls (MGD)	BRvitrea	**0.08**	Decline	3.03	36.83	0.016	0.958	0.01	0.06	1.03
Outfalls (MGD)	CMlaevis	**0.01**	Decline	6.17	41.67	0.004	0.992	0.01	0.01	0.10
Outfalls (MGD)	ALpelluc	**0.01**	Decline	3.30	33.65	0.024	0.990	0.01	0.06	0.94
Outfalls (MGD)	CMdelcat	**0.58**	Decline	4.49	51.23	0.004	1.000	0.06	0.69	3.26
Outfalls (MGD)	SYacus	**0.32**	Decline	3.77	40.29	0.012	0.990	0.01	0.14	0.81
Outfalls (MGD)	GOclavat	**0.58**	Decline	4.63	52.23	0.004	0.994	0.06	0.44	3.51
Outfalls (MGD)	NAradios	**0.06**	Decline	3.32	30.31	0.012	0.992	0.01	0.08	0.81
Outfalls (MGD)	FRcapuci	**0.44**	Decline	3.70	52.92	0.008	0.980	0.01	0.18	1.03
Outfalls (MGD)	BApardxa	**0.01**	Decline	3.07	23.08	0.036	0.960	0.01	0.01	0.08
Outfalls (MGD)	CMaffins	**0.18**	Decline	4.36	47.62	0.004	0.990	0.01	0.10	0.94
Outfalls (MGD)	GOangstt	**0.94**	Decline	3.93	50.14	0.012	0.994	0.08	0.58	3.26
Outfalls (MGD)	CMkolbei	**0.94**	Decline	3.31	45.56	0.012	0.986	0.01	0.58	2.17
Outfalls (MGD)	EYevergl	**0.06**	Decline	5.35	64.86	0.004	1.000	0.01	0.18	1.22
Outfalls (MGD)	EYmicroc	**1.22**	Decline	4.22	58.09	0.004	0.994	0.03	0.81	3.26
Outfalls (MGD)	ACbiasol	**0.01**	Decline	4.95	67.93	0.004	0.998	0.01	0.10	3.26

Outfalls (MGD)	NAcryten	**0.01**	Decline	4.42	52.57	0.004	0.982	0.01	0.01	0.32
Outfalls (MGD)	DEkuetzi	**0.08**	Decline	6.02	71.15	0.004	0.998	0.01	0.08	0.58
Outfalls (MGD)	AHminuti	**0.08**	Decline	7.17	77.47	0.004	1.000	0.01	0.18	2.28
Outfalls (MGD)	ECsilesi	**3.51**	Decline	3.56	64.13	0.008	0.984	0.32	2.17	4.15
Outfalls (MGD)	CHRsp	**5.69**	Increase	6.83	88.44	0.004	1.000	0.44	4.55	6.41
Outfalls (MGD)	SCEquadr	**4.15**	Increase	8.62	42.86	0.008	0.964	3.51	4.55	6.41
Outfalls (MGD)	AMveneta	**6.41**	Increase	7.35	93.02	0.004	0.994	1.22	4.92	6.41
Outfalls (MGD)	GMgrovei	**6.41**	Increase	4.32	61.00	0.024	0.976	0.44	5.69	6.41
Outfalls (MGD)	ROabbre	**2.17**	Increase	4.51	33.33	0.012	0.986	0.58	2.17	4.55
Outfalls (MGD)	FRellptc	**3.26**	Increase	3.48	39.10	0.012	0.958	0.32	3.26	6.41
Outfalls (MGD)	TEmusica	**0.32**	Increase	3.44	31.58	0.024	0.992	0.08	0.44	4.92
Outfalls (MGD)	NIcombal	**0.32**	Increase	4.44	36.84	0.004	0.998	0.10	0.58	2.17
Outfalls (MGD)	NIangtu	**0.81**	Increase	4.03	31.25	0.004	0.994	0.18	0.87	3.51
Outfalls (MGD)	GMlinfor	**0.32**	Increase	5.33	47.37	0.004	1.000	0.08	0.81	6.41
Outfalls (MGD)	NIangust	**0.06**	Increase	4.00	39.13	0.008	0.992	0.01	0.18	1.03
Outfalls (MGD)	FAtener2	**1.22**	Increase	4.36	35.64	0.008	0.982	0.18	0.87	3.51
Outfalls (MGD)	NIsolita	**0.08**	Increase	4.36	45.45	0.008	1.000	0.01	0.10	1.22
Outfalls (MGD)	PRlaevis	**5.69**	Increase	4.38	80.36	0.004	0.994	0.44	3.89	6.41
Outfalls (MGD)	AMpedcls	**0.81**	Increase	5.75	57.62	0.004	0.996	0.03	0.44	1.22
Outfalls (MGD)	HIhunga	**1.22**	Increase	4.02	47.88	0.004	0.968	0.01	0.94	3.51
Outfalls (MGD)	NAsancru	**0.44**	Increase	6.47	55.56	0.004	1.000	0.10	0.58	2.17
Outfalls (MGD)	DIconfer	**3.26**	Increase	5.61	76.18	0.004	1.000	0.18	2.17	3.63
Outfalls (MGD)	GOpumilu	**1.22**	Increase	5.18	63.21	0.004	0.980	0.32	1.03	3.51
Outfalls (MGD)	NArecens	**0.32**	Increase	5.97	62.84	0.004	0.980	0.06	0.32	1.03
Outfalls (MGD)	REsinuta	**0.58**	Increase	3.53	59.25	0.004	0.994	0.01	0.32	2.17
Outfalls (MGD)	CCplacen	**0.18**	Increase	5.72	69.16	0.004	1.000	0.06	0.32	0.94
Outfalls (MGD)	GOparvul	**0.18**	Increase	3.42	58.98	0.012	0.972	0.01	0.06	0.58
Chloride (mg/L)	GLHsp	**18.50**	Decline	2.41	57.17	0.004	0.982	13.50	18.00	35.00
Chloride (mg/L)	MERglauc	**19.50**	Decline	7.94	77.50	0.004	0.998	18.00	20.50	26.50

Chloride (mg/L)	OSCsp	**21.50**	Decline	3.29	55.89	0.004	0.968	18.00	24.00	72.50
Chloride (mg/L)	GOmaclau	**20.50**	Decline	3.21	29.41	0.020	0.994	11.00	18.50	24.00
Chloride (mg/L)	GOintvib	**26.00**	Decline	3.87	35.00	0.012	0.992	18.00	21.50	30.00
Chloride (mg/L)	NAstroem	**20.50**	Decline	6.70	58.82	0.004	1.000	17.00	19.50	26.00
Chloride (mg/L)	BRvitrea	**20.50**	Decline	4.83	45.89	0.004	0.998	18.00	20.50	28.50
Chloride (mg/L)	CMlaevis	**18.00**	Decline	5.33	41.67	0.004	0.998	11.50	15.00	19.50
Chloride (mg/L)	NIampoid	**20.50**	Decline	3.12	33.60	0.012	0.992	13.50	19.00	28.50
Chloride (mg/L)	ALpelluc	**26.00**	Decline	3.32	30.00	0.004	0.956	10.50	24.00	30.10
Chloride (mg/L)	NAcrypto	**30.00**	Decline	2.41	26.09	0.020	0.964	13.50	24.00	31.00
Chloride (mg/L)	CMdelcat	**20.50**	Decline	5.18	57.82	0.004	1.000	15.00	20.50	30.00
Chloride (mg/L)	SYacus	**20.50**	Decline	2.75	35.21	0.020	0.984	18.00	24.00	60.50
Chloride (mg/L)	GOclavat	**24.00**	Decline	4.53	51.07	0.008	0.990	13.50	18.50	26.00
Chloride (mg/L)	GOgracil	**69.00**	Decline	3.72	53.57	0.004	0.998	13.50	31.00	73.50
Chloride (mg/L)	NAradios	**12.50**	Decline	4.30	45.70	0.004	0.998	11.00	15.00	21.50
Chloride (mg/L)	FRcapuci	**26.00**	Decline	5.01	59.78	0.004	0.994	13.50	21.50	31.00
Chloride (mg/L)	CMaffins	**18.00**	Decline	5.90	56.70	0.004	0.996	11.50	17.00	26.00
Chloride (mg/L)	GOangstt	**12.50**	Decline	5.08	70.05	0.004	1.000	11.50	17.00	26.05
Chloride (mg/L)	CMkolbei	**31.00**	Decline	4.88	50.00	0.008	1.000	11.00	20.50	35.00
Chloride (mg/L)	EYevergl	**19.00**	Decline	6.64	75.32	0.004	1.000	15.00	19.00	28.65
Chloride (mg/L)	EYmicroc	**26.00**	Decline	5.56	62.75	0.004	0.996	18.00	21.50	30.00
Chloride (mg/L)	PIgibba	**35.00**	Decline	2.71	36.00	0.040	0.976	11.00	28.50	60.50
Chloride (mg/L)	ACbiasol	**26.00**	Decline	5.57	62.60	0.004	0.992	15.00	20.50	28.50
Chloride (mg/L)	DEkuetzi	**21.50**	Decline	4.18	60.35	0.004	0.996	18.00	21.50	60.50
Chloride (mg/L)	AHminuti	**24.00**	Decline	6.05	69.10	0.004	1.000	19.00	24.00	46.50
Chloride (mg/L)	ECsilesi	**17.00**	Decline	4.26	66.01	0.004	0.998	13.50	18.50	35.00
Chloride (mg/L)	SYulna	**94.50**	Decline	6.56	94.12	0.004	1.000	69.00	92.50	100.50
Chloride (mg/L)	ANBsp	**31.00**	Increase	2.83	21.43	0.048	0.964	24.00	31.00	69.00
Chloride (mg/L)	CHRsp	**24.00**	Increase	4.69	42.11	0.004	1.000	19.50	26.00	46.50
Chloride (mg/L)	SCEquadr	**69.00**	Increase	4.52	30.00	0.016	0.968	31.00	72.50	92.50
Chloride (mg/L)	NAsubmin	**31.00**	Increase	3.01	21.43	0.032	0.966	26.50	35.00	92.50

Chloride (mg/L)	AMveneta	**60.50**	Increase	6.55	45.45	0.004	0.994	31.00	72.50	92.50
Chloride (mg/L)	ROabbre	**92.50**	Increase	8.96	59.02	0.004	0.982	69.00	86.00	94.50
Chloride (mg/L)	FRellptc	**46.50**	Increase	5.30	48.22	0.004	1.000	26.00	46.50	86.00
Chloride (mg/L)	NItropic	**26.50**	Increase	2.95	23.53	0.036	0.978	20.50	28.50	73.85
Chloride (mg/L)	NIcombal	**26.50**	Increase	4.81	41.18	0.004	1.000	24.00	46.50	94.50
Chloride (mg/L)	NIangtu	**94.50**	Increase	4.28	43.76	0.012	0.982	20.50	69.00	100.50
Chloride (mg/L)	SUbreb	**94.50**	Increase	5.49	45.30	0.008	0.960	26.00	77.00	100.50
Chloride (mg/L)	AMsabina	**18.50**	Increase	4.14	45.83	0.008	0.996	18.00	20.50	35.00
Chloride (mg/L)	GMlinfor	**35.00**	Increase	2.96	37.33	0.020	0.972	18.00	28.50	72.50
Chloride (mg/L)	TYapicul	**31.00**	Increase	4.05	43.11	0.008	0.994	20.50	35.00	94.50
Chloride (mg/L)	FAtener2	**92.50**	Increase	5.01	53.29	0.012	1.000	26.00	69.00	100.50
Chloride (mg/L)	PCplacen	**26.00**	Increase	5.31	44.44	0.008	1.000	21.50	30.00	86.00
Chloride (mg/L)	PRlaevis	**46.50**	Increase	7.64	69.18	0.004	1.000	26.50	35.00	72.50
Chloride (mg/L)	AMpedcls	**69.00**	Increase	3.50	58.66	0.012	0.986	17.00	46.50	86.00
Chloride (mg/L)	AHexigum	**21.50**	Increase	4.87	62.19	0.004	0.998	18.50	26.00	60.50
Chloride (mg/L)	HIhunga	**92.50**	Increase	6.46	87.18	0.004	1.000	19.00	73.50	94.50
Chloride (mg/L)	PTlanceo	**31.00**	Increase	3.65	40.25	0.008	0.956	19.00	30.00	72.50
Chloride (mg/L)	NAsancru	**20.50**	Increase	2.97	37.20	0.032	0.974	17.00	24.00	72.50
Chloride (mg/L)	DIconfer	**28.50**	Increase	6.31	71.71	0.004	1.000	18.50	26.00	35.00
Chloride (mg/L)	NIincons	**20.50**	Increase	5.16	67.15	0.004	1.000	18.00	21.50	73.85
Chloride (mg/L)	NIfrustu	**31.00**	Increase	3.18	53.80	0.020	0.954	20.50	35.00	94.50
Chloride (mg/L)	NArostel	**26.00**	Increase	2.81	39.65	0.028	0.986	17.00	26.00	86.00
Chloride (mg/L)	NArecens	**24.00**	Increase	4.54	55.41	0.008	0.978	18.00	24.00	77.00
Chloride (mg/L)	CYmenegh	**92.50**	Increase	4.04	75.49	0.004	0.986	20.50	35.00	94.50
Chloride (mg/L)	GOparvul	**19.50**	Increase	2.73	56.39	0.008	0.964	18.00	30.00	86.00

Appendix A7. Taxa-specific results from Threshold Indicator Taxa Analysis (TITAN) on **fish species composition** in response to nutrient and nutrient-related stressors among 38 sites in **Ecoregion 29** during summer 2008. Only fish species that showed significant threshold declines or increases in response to predictors are included in this table. The observed (*Obs*) threshold value of the predictor for each taxon is shown in bold, whereas lower (*10%*), middle (*50%*), and upper (*90%*) quantiles of 1,000 bootstraps represent measures of uncertainty around the observed threshold. *Z* represents the standardized indicator score from TITAN (larger numbers = stronger threshold response), *IndVal* is the unstandardized indicator score (scaled from 0-100%, with 100=perfect indicator). *Purity* is the relative consistency of the response direction among the 1,000 bootstraps (*purity* > 0.95 is significant). *P-value* is the likelihood of getting an equal or larger IndVal if the score were computed with random shuffling of the observed data (*P*<0.05 is significant). See Appendix A6 for full species names corresponding to *Taxon IDs*.

								Bootstrap threshold quantiles		
Predictor	Taxon ID	Threshold (obs)	Response > obs.	z	IndVal	P	Purity	10%	50%	90%
TP (ug/L)	CAMPANOM	**24.2**	Decline	4.6	66.6	0.004	0.980	19.7	27.8	44.7
TP (ug/L)	CYPRVENU	**17.0**	Decline	2.5	59.0	0.016	0.972	14.6	18.2	368.3
TP (ug/L)	ETHESPEC	**19.7**	Decline	5.8	73.8	0.004	1.000	17.0	26.8	44.7
TP (ug/L)	LEPOGULO	**14.3**	Decline	2.2	49.8	0.044	0.940	12.4	19.7	52.1
TP (ug/L)	LEPOMACR	**61.9**	Decline	4.0	61.4	0.004	0.982	34.2	69.8	598.3
TP (ug/L)	CARPCARP	**52.1**	Increase	3.8	33.6	0.008	0.980	28.9	52.1	770.3
TP (ug/L)	CYPRCARP	**187.5**	Increase	5.7	59.1	0.004	0.990	34.2	125.1	932.2
TP (ug/L)	CYPRLUTR	**21.4**	Increase	4.9	67.0	0.004	0.996	16.9	27.8	52.1
TP (ug/L)	LEPIOSSE	**34.2**	Increase	3.9	40.2	0.004	0.962	19.7	40.7	187.5
TP (ug/L)	PIMEVIGI	**21.4**	Increase	4.2	63.3	0.004	0.986	14.6	26.8	44.7
Pasture (%)	CYPRVENU	**12.3**	Decline	4.4	68.0	0.004	1.000	3.8	11.5	13.2
Pasture (%)	CAMPANOM	**7.1**	Decline	3.8	65.7	0.004	0.998	2.4	6.9	12.3
Pasture (%)	ETHESPEC	**4.5**	Decline	5.2	71.8	0.004	0.998	2.8	4.5	7.8
Pasture (%)	MOXOCONG	**8.4**	Decline	4.0	50.0	0.004	0.996	3.1	7.1	8.8
Pasture (%)	NOTRVOLU	**8.6**	Decline	4.1	51.9	0.008	0.990	2.6	8.4	9.8
Pasture (%)	ICTAPUNC	**10.9**	Decline	4.4	72.3	0.004	0.956	7.8	9.8	12.6

Pasture (%)	CYPRLUTR	**1.6**	Increase	6.1	74.5	0.004	1.000	0.8	1.8	3.3
Pasture (%)	DOROCEPE	**8.8**	Increase	5.6	55.8	0.004	1.000	3.1	8.6	11.5
Pasture (%)	PIMEVIGI	**3.3**	Increase	5.2	66.7	0.004	1.000	0.9	2.8	5.8
Pasture (%)	CARPCARP	**3.8**	Increase	4.4	33.3	0.008	0.996	3.1	5.8	12.3
Pasture (%)	POMOANNU	**4.5**	Increase	3.8	29.4	0.008	0.994	2.8	4.5	8.6
Pasture (%)	LYTHUMBR	**9.8**	Increase	6.5	44.4	0.004	0.980	8.6	10.9	13.2
Pasture (%)	NOTUGYRI	**6.9**	Increase	2.7	20.0	0.056	0.952	3.3	7.1	8.8
Outfalls (MGD)	CAMPANOM	**0.3**	Decline	4.9	67.9	0.004	0.984	0.0	0.3	0.9
Outfalls (MGD)	ETHESPEC	**0.3**	Decline	5.3	71.4	0.004	0.990	0.0	0.3	0.9
Outfalls (MGD)	FUNDNOTA	**2.2**	Decline	4.3	64.0	0.004	0.986	0.8	2.2	4.1
Outfalls (MGD)	LEPOCYAN	**0.8**	Decline	3.5	56.1	0.004	0.948	0.2	1.2	5.7
Outfalls (MGD)	LEPOMACR	**2.2**	Decline	3.8	60.7	0.004	0.982	0.4	1.2	3.6
Outfalls (MGD)	CYPRCARP	**4.1**	Increase	3.1	46.2	0.008	0.966	0.1	3.5	5.7
Outfalls (MGD)	CYPRLUTR	**0.4**	Increase	4.5	66.4	0.004	0.996	0.0	0.2	1.0
Outfalls (MGD)	DOROCEPE	**0.4**	Increase	2.8	30.7	0.024	0.910	0.0	0.9	5.7
Outfalls (MGD)	ICTAPUNC	**3.5**	Increase	3.2	64.5	0.008	0.902	0.8	3.5	4.5
Outfalls (MGD)	LEPIOSSE	**0.6**	Increase	3.8	40.2	0.016	0.944	0.1	0.8	3.2
Outfalls (MGD)	PIMEVIGI	**0.4**	Increase	4.7	66.4	0.004	0.952	0.0	0.3	1.0
Outfalls (MGD)	PYLOOLIV	**3.5**	Increase	5.0	72.9	0.004	0.998	0.8	2.2	3.6
Mud-silt (%)	CAMPANOM	**0.0**	Decline	4.0	64.6	0.008	0.942	0.0	3.8	12.5
Mud-silt (%)	CYPRVENU	**1.5**	Decline	3.1	58.6	0.012	0.996	0.0	8.8	17.8
Mud-silt (%)	ICTAPUNC	**15.0**	Decline	3.9	67.4	0.004	0.976	7.3	15.0	17.0
Mud-silt (%)	LEPOCYAN	**7.3**	Decline	3.5	56.4	0.012	0.970	0.9	8.8	18.2
Mud-silt (%)	NOTRVOLU	**7.5**	Decline	2.5	43.5	0.032	0.950	0.0	6.0	15.4
Mud-silt (%)	CYPRLUTR	**6.0**	Increase	3.8	64.1	0.004	0.982	0.2	3.8	8.8
Mud-silt (%)	LEPIOSSE	**15.0**	Increase	3.8	47.9	0.012	0.926	2.2	15.0	18.2

Mud-silt (%)	LYTHUMBR	**16.0**	Increase	6.6	50.0	0.004	0.992	12.5	16.0	18.2
Mud-silt (%)	PIMEVIGI	**1.5**	Increase	3.7	63.1	0.004	0.992	0.2	2.5	12.5
Chloride (mg/L)	AMEINATA	**12.0**	Decline	3.3	68.4	0.004	0.980	11.0	17.0	26.5
Chloride (mg/L)	CAMPANOM	**17.0**	Decline	4.5	68.7	0.004	1.000	15.0	19.5	86.0
Chloride (mg/L)	ETHESPEC	**30.0**	Decline	4.6	69.2	0.004	1.000	17.0	24.0	60.5
Chloride (mg/L)	FUNDNOTA	**60.5**	Decline	3.7	61.0	0.004	0.974	19.0	46.5	73.5
Chloride (mg/L)	LEPOGULO	**19.5**	Decline	3.5	49.5	0.016	0.962	15.0	19.0	28.5
Chloride (mg/L)	LEPOMACR	**92.5**	Decline	4.6	66.4	0.004	0.998	30.0	77.0	100.5
Chloride (mg/L)	CARPCARP	**46.5**	Increase	6.2	50.0	0.004	1.000	28.5	46.5	73.5
Chloride (mg/L)	CYPRCARP	**30.0**	Increase	3.9	40.6	0.008	0.990	18.5	35.0	94.5
Chloride (mg/L)	CYPRLUTR	**13.5**	Increase	7.9	88.1	0.004	1.000	11.5	13.5	18.0
Chloride (mg/L)	PIMEVIGI	**13.5**	Increase	6.9	86.3	0.004	1.000	11.5	13.5	19.0
Chloride (mg/L)	PYLOOLIV	**19.5**	Increase	3.5	58.5	0.004	0.936	17.0	20.5	61.4